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LETTER

Intermittent Dose Schedule of Mitomycin C (NSC-26980) in Solid Tumors

In the June 1971 issue of *Cancer Chemotherapy Reports*, Sutow et al (1), quoting Carter (2), stated that they were "unable to find reference to clinical studies of mitomycin C using intermittent dose regimens." In 1964, we published results of a non-controlled clinical trial with mitomycin C in adult patients who had solid tumors (3,4). We found that intermittent large doses (20–50 mg given intravenously every 3–5 weeks) produced much less hematologic toxicity than the usual dose schedule (2 mg daily to 10 mg 3 times a week). Leukopenia (<3000 leukocytes/mm^3) was observed in seven of 22 patients receiving the intermittent schedule and in 22 of 28 patients receiving the usual dose schedule ($P = 0.001$). Thrombocytopenia (platelets $<100{,}000$/mm^3) was also less frequent and less severe with large intermittent doses. With doses up to 30 mg, there was no cumulative toxicity when the interval between doses was 3–5 weeks.

The therapeutic responses (significant decrease of measurable lesions for a duration of not less than 1 month) were slightly more frequent with the intermittent regimen (five "definite objective responses" among 28 evaluable cases) than with the usual "continuous" dose schedule (six among 37 evaluable cases). There were also five "questionable objective responses" (decrease of measurable lesions for less than 1 month, or marked decrease of non-measurable lesions) in the first group and three in the second group. Objective responses were observed in lung cancer (four of 16), head and neck epidermoid carcinoma (seven of 23), gastrointestinal glandular carcinoma (three of eight), and other miscellaneous tumors (five of 18).

The conclusion of one of our papers (5) was as follows: "Among the patients treated intravenously, the objective responses were more frequent and toxicity much less important in the subgroup of patients who received the massive (intermittent) dosage. This may represent an advance in the treatment with this drug and one of the few examples of dissociation between therapeutic efficiency and side-effects." Since that time we have routinely treated patients with intra-oral epidermoid carcinoma, not amenable to or recurring after surgical treatment and radiation therapy, with intermittent doses of 20–25 mg/m^2 iv every 3 weeks, producing very moderate toxicity and encouraging antitumor effects.

REFERENCES

1. Sutow, W. W., Wilbur, J. R., Vietti, T. J., et al. Evaluation of dosage schedules of mitomycin C (NSC-26980) in children. Cancer Chemother Rep 53:285–289, 1971.
2. Carter, S. K. Mitomycin C (NSC-26980): clinical brochure. Cancer Chemother Rep Part 3, vol 1:99–114, 1968.
3. Kenis, Y., and Stryckmans, P. Action de la mitomycine C sur l'hématopoiese dans le cancer en phase avancée. Nouv Rev Fr Hematol 4:15–30, 1964.
4. Kenis, Y., and Stryckmans, P. Action de la mitomycine C dans 65 cas de tumeurs malignes. Comparaison de l'effet de doses faibles, répétées et de doses "massives." Chemotherapia 8:114–138, 1964.
5. Kenis, Y., Stryckmans, P., and Lebrun, J. Clinical trial with mitomycin C in patients with solid tumors. *In* Chemotherapy of Cancer (Plattner, P. A., ed). Amsterdam, Elsevier Publishing Company, 1964, pp 153–161.

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February 2, 1972

Activity of Adriamycin (NSC-123127) and Daunomycin (NSC-82151) Against Mouse Mammary Carcinoma [1]

A. Di Marco, L. Lenaz, A. M. Casazza, and B. M. Scarpinato [2,3]

SUMMARY

This report compares the activities of daunomycin and adriamycin (14-hydroxydaunomycin) against spontaneous and transplanted mammary carcinoma in C3H mice. The antibiotics were administered intravenously according to different schedules of treatment. After two cycles of six daily doses of each antibiotic had been given to mice bearing spontaneous mammary carcinoma, a marked decrease in tumor size, accompanied by loss of body weight, was observed in both daunomycin- and adriamycin-treated groups. When a schedule of two cycles of six treatments every other day was used, however, a significant reduction in tumor growth rate was noted only in adriamycin-treated mice. Against transplanted mammary carcinoma, adriamycin was constantly more effective than daunomycin at the doses tested. The administration of adriamycin every other day (thus, over a long period of time) resulted in a reduction of tumor takes and in a high number of long-term survivors.

[Cancer Chemother Rep 56:153–161, 1972]

The physicochemical properties of adriamycin (14-hydroxydaunomycin), as well as preliminary data concerning its toxicity and antitumor activity, have been reported (1–3).

The antitumor effect of adriamycin was greater than that of daunomycin on Ehrlich ascites carcinoma, Oberling-Guérin-Guérin myeloma, solid Sarcoma 180 (S180), nitrosomethylurea-induced lymphosarcoma (3), L1210 and P388 leukemias (4), and Moloney sarcoma virus-induced rhabdomyosarcoma (5). The therapeutic index, defined as the ratio between the minimal effective dose (minimal dose giving a 90% inhibition of tumor growth) and the maximal tolerated dose (LD10), is 1.21 for adriamycin and 0.67 for daunomycin in S180-bearing Swiss mice (3).

The reason for this difference between the two antibiotics cannot at present be accounted for by supposing a difference in mechanisms of action, since adriamycin and daunomycin are quite similar in DNA binding affinity, DNA synthesis inhibition, and effect on in vitro cell cultures (6,7).

This paper compares the antitumor activities of adriamycin and daunomycin against spontaneous and transplanted mouse mammary carcinoma, and describes an attempt to define a suitable schedule for treating these tumor systems with the two antibiotics.

MATERIALS AND METHODS

Mice.—Inbred C3H/He Dp mice were obtained from the breeding colony of Dr. G. Della Porta (Department of Experimental Oncology

[1] Received May 28, 1971; revised Dec 6, 1971.

[2] Divisione di Oncologia Sperimentale B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.

[3] The authors wish to thank Mrs. T. Comisso for her valuable technical assistance.

A, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy) for use in this study.

Tumors.—Female mice with spontaneously occurring mammary carcinomas were routinely removed from the breeding colony and were used in studies on spontaneous mammary carcinoma. The rate of appearance of mammary tumors in this mouse line is 64%; the average age of the mice at tumor appearance is 7.5 months (G. Parmiani and G. Della Porta, personal communication).

Transplantable mammary carcinomas were obtained by subcutaneous inoculation with a cell suspension from spontaneous mammary carcinomas. The tumors used were at the third, fourth, fifth, or sixth transplant stage. Mice received injections of about 2×10^7 cells, a dose which gives 100% of tumor takes and kills the animals in about 60 days. In our system, the minimum number of cells leading to 100% tumor takes and progressive tumor growth is 1×10^6 cells/mouse (evaluation 3 months after transplant).

Each experimental group included at least ten randomized animals (five males and five females) about 50 days old, weighing from 25 g (females) to 30 g (males).

Measuring tumor growth.—The growth rate of tumors was determined twice a week by measuring the maximal and minimal diameters of the tumor with calipers; the volume was calculated as (the minimal diameter)$^2 \times$ (the maximal diameter). The curves indicating tumor growth in the experiments on transplanted carcinoma were drawn according to the "moving average method" (8).

Treatment.—Daunomycin and adriamycin were dissolved in Ringer's solution and were administered intravenously (iv) in volumes of 10 ml/kg/mouse, at doses corresponding to equal fractions (1/8, 1/10) of the acute LD50 (3). The higher dose is the maximal tolerated dose after treatment for 6–8 consecutive days (unpublished results from this laboratory). In one experiment an intermediate dose of adriamycin was used also.

RESULTS

Activity Against Spontaneous Mammary Carcinoma

Impairment of the growth of primary mammary tumors in C3H mice by daunomycin and adriamycin is indicated by the data shown in table 1 and figure 1. When administered daily, both adriamycin and daunomycin caused a significant decrease in tumor size, both during and immediately after the treatment. This effect was accompanied by a loss of body weight equal to 11%–17%. Later, when treatment was stopped, a resumption of tumor growth was observed, the tumors developing parallel to the tumors in control animals, until the mice died.

At the dose used, treatment every second day with daunomycin caused only a 46% inhibition of the tumor growth rate which was accompanied by a loss of body weight similar to that

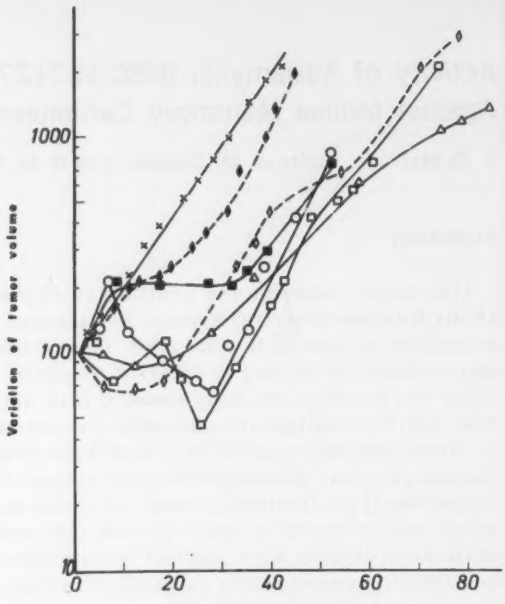

FIGURE 1.—Activity of adriamycin and daunomycin against spontaneous mammary carcinoma. Variation of tumor volume from the value at beginning of treatment (Day 0), made equal to 100. Open symbols (○, □, △, ◇): treatment daily; Days 1–7, 12–17. Filled symbols (■, ◆): treatment every other day; Days 1–11, 17–27.

Symbols		*Drug*	*Dose*
×——×	=	Controls	
◇ - - - - ◇	=	Daunomycin	3.25 mg/kg/day
◆ - - - - ◆	=	Daunomycin	3.25 mg/kg/day
○——○	=	Adriamycin	2 mg/kg/day
□——□	=	Adriamycin	2.25 mg/kg/day
■——■	=	Adriamycin	2.25 mg/kg/day
△——△	=	Adriamycin	2.5 mg/kg/day

TABLE 1.—Effects of daunomycin and adriamycin on spontaneous mammary carcinoma in C3H female mice

Treatment				Average animal weight				Average tumor volume			
						Percent				Percent	
Treatment schedule (days after transplant)	Drug	Mg/kg/day	No. of animals	Before treatment (g±SE)	At end of treatment (g±SE)	a*	b†	Before treatment (cm^3±SE)	At end of treatment (cm^3±SE)	a*	b†
Controls	—	—	23	33.2±0.6	33.7±1.0	101.8	—	2.1 ±0.5	6.4 ±1.2	368.0	—
Daily; Days 1–6, 12–17	Daunomycin	3.25	11	31.3±0.9	28.2±0.7	91.0	10.6	2.2 ±0.9	1.7 ±0.5	96.2‡	74.6
	Adriamycin	2.0	16	34.3±1.0	28.7±0.6	84.1	17.4	2.5 ±0.9	1.9 ±0.6	115.1‡	69.6
	Adriamycin	2.25	11	32.6±0.8	29.2±0.4	85.7	11.9	0.25±0.04	0.17±0.04	95.9‡	74.6
	Adriamycin	2.5	11	31.1±0.5	28.0±0.6	90.3	11.3	1.2 ±0.2	1.0 ±0.1	96.8‡	74.4
Controls	—	—	21	34.5±0.7	37.1±0.8	108.0	—	2.3 ±0.5	12.4 ±1.7	860.1	—
Every other day; Days 1–11, 17–27	Daunomycin	3.25	9	34.1±1.7	30.1±1.1	89.6	17.0	1.6 ±0.5	4.6 ±1.1	560.2	34.9
	Adriamycin	2.25	11	34.3±1.3	30.6±0.9	90.0	16.7	3.2 ±0.9	4.1 ±1.0	205.5‡§	76.1

*a: Values at end of treatment compared with values before treatment. Average of percents of individual variations.
†b: Percent reduction as compared to controls. Average of percents of individual variations.
‡Significantly different from controls at the 99% level ("Student's t" test).
§Significantly different from daunomycin-treated mice at the 99% level ("Student's t" test).

observed after daily treatments; treatment with adriamycin according to the same schedule caused a much greater inhibition of tumor growth rate (76%) and a similar effect on body weight. Statistical analysis demonstrated that animals treated with adriamycin had significantly reduced tumor growth, as compared with untreated animals (controls) and daunomycin-treated animals.

Activity Against Transplanted Mammary Carcinoma

Daunomycin and adriamycin, when administered for 8 consecutive days starting on the first or on the tenth day after the transplant (fig 2), had little effect on tumor growth. Parallel to the slight inhibition of tumor growth, significant change in the average survival time of treated animals in comparison with the control animals was obtained only in animals treated from the first to the eighth day after transplant (table 2).

When the antibiotics were administered in three cycles of 7, 3, and 3 days of treatment, a strong inhibition of tumor development was observed in the groups treated with adriamycin; treatment with daunomycin was less effective (fig 3). Increase in the average survival time was significant only after treatment with adriamycin at the higher dose, and only when the total population was considered (table 2). In general, grossly visible tumor metastases observed in the lungs, spleen, liver, kidneys, or testicles were more frequent in the treated animals than in the control animals, where metastases occur very rarely. Moreover, in the treated animals, toxic effects of daunomycin and adriamycin were observed, including hemorrhagic degeneration of the intestinal mucosa, and spleen and liver weight reduction.

On the basis of these results, it was supposed that the schedules used were unsuitable for the treatment of this experimental tumor, which grows rather slowly soon after it has been transplanted, and that, early in the course of treatment, the toxic effects of the antibiotics would be more apparent than the antitumor effects. Therefore, a different schedule of treatment was used; the total dose administered was the same as in the last experiment described but

TABLE 2.—Daunomycin and adriamycin activity against transplanted mammary carcinoma: daily treatment

Treatment			Average survival time (days+SE)*		
Treatment schedule (days after transplant)	Drug	Mg/kg/day	Males	Females	Total
Controls	—	—	46.0±5.9	54.6±7.1	49.8±4.5
Daily: Days 1–8	Daunomycin	2.6	59.8±4.0	62.2±3.1	60.9±2.5*
	Daunomycin	3.25	68.0±7.7	65.6±6.2	66.9±4.8*
	Adriamycin	2.0	71.3±5.8*	59.4±3.9	65.9±3.9*
	Adriamycin	2.5	59.7±4.1	52.4±10.6	56.4±5.1
Controls	—	—	50.5±11.5	70.0±5.2	60.2±6.7
Daily: Days 10–17	Daunomycin	2.6	60.5±4.9	71.8±8.9	66.2±5.1
	Daunomycin	3.25	49.3±6.7	55.0±2.8	52.2±3.6
	Adriamycin	2.0	59.5±5.5	61.3±4.6	60.4±3.4
	Adriamycin	2.5	63.7±2.5	64.3±5.9	64.0±3.1
Controls	—	—	72.8±1.8	70.6±6.0	71.7±3.0
Daily: Days 1–6, 12–14, 17–19	Daunomycin	2.6	76.8±5.2	66.8±3.5	71.8±3.4
	Daunomycin	3.25	59.6±8.5	78.0±3.7	68.8±5.4
	Adriamycin	2.0	72.8±6.1	88.8±6.6	80.8±5.0
	Adriamycin	2.5	81.4±4.1	90.8±8.1	86.1±4.5*

* "Student's t" test was calculated by comparing controls with treated groups. The probability that the differences between the two groups were not due to chance is indicated at the 95%(*) level. "Average survival time" was computed from the time of transplant.

the antibiotics were administered every other day and, consequently, over a longer period of time.

Results, reported in figure 4 and in table 3, show that, using this treatment schedule (ie, treatment on alternate days), it is possible to obtain a strong inhibition of tumor growth, as well as a significant increase in the average survival time. The activity of adriamycin was much greater than that of daunomycin. In the animals treated with daunomycin the increase in the average survival time was more noticeable in males than in females. In the animals treated with adriamycin the transplanted tumor did not develop in most cases. Late tumors appeared in 11 of 14 animals 7 or more months from the time of transplant, in sites other than that of the original inoculation; these tumors were thought to be spontaneous and were not studied. In the daunomycin-treated animals that died with enlarged tumor, no metastases were observed.

DISCUSSION

Primary tumors in mice, growing autochthonously in their host, are considered similar to human tumors with respect to their mode of occurrence, but their growth (as transplants) in highly inbred animals results in minimal development of differences in their properties due to host immunologic mechanisms. Mouse mammary tumor, in particular, is considered a useful model for the study of human mammary cancer.

Treatment of animals with daunomycin and adriamycin resulted in a decrease of tumor growth rate during and shortly after treatment. The loss of body weight observed in treated animals can be considered an index of the toxicity induced by the drugs (9). A definite correlation between body weight reduction and tumor growth inhibition has been demonstrated in C3H mice with mammary carcinoma. Mice treated with reserpine (0.3 mg/kg/day for 11 days ip) had a 15% body weight reduction (secondary to starvation) and a 42% tumor growth inhibition when compared with controls (10). In our studies, weight losses in daunomycin- and adriamycin-treated mice were comparable when the antibiotics were administered every second day, however, adriamycin seemed to be significantly more effective than daunomycin in inhibiting tumor growth.

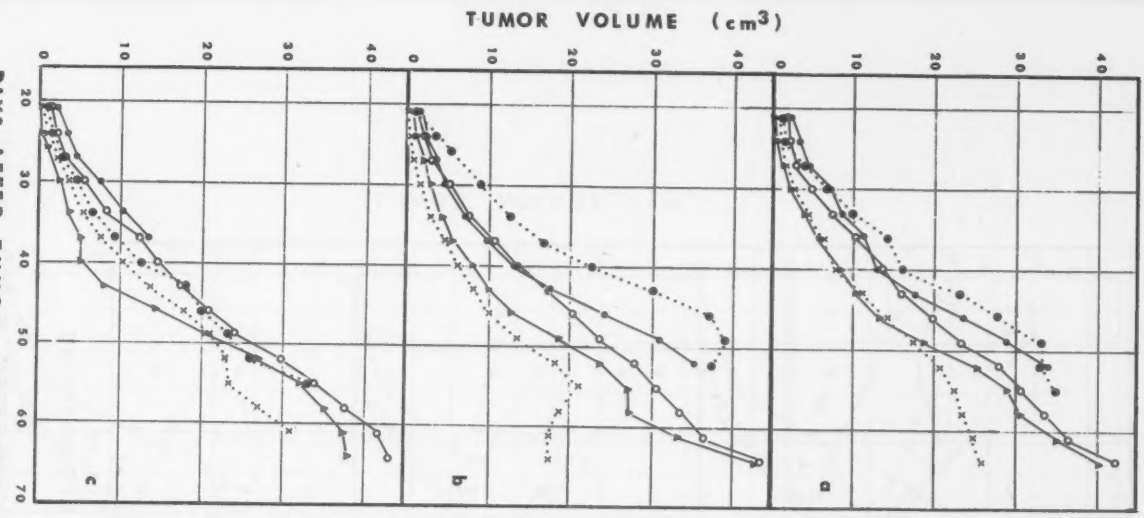

FIGURE 2.—Activity of adriamycin and daunomycin against transplanted mammary carcinoma. Daily treatment from the tenth to the 17th day after the transplant. a = Whole population; b = females; c = males. ●—● = Controls. ○—○ = Daunomycin, 2.6 mg/kg/day. ● . . . . ● = Daunomycin, 3.25 mg/kg/day. △——△ = Adriamycin, 2.0 mg/kg/day. × . . . . × = Adriamycin, 2.5 mg/kg/day.

TABLE 3.—Daunomycin and adriamycin activity against transplanted mammary carcinoma: treatment on alternate days

Treatment			Males			Females			Total		
Treatment schedule (days after transplant)	Drug	Mg/kg/day	Average survival time (days±SE)*†	Tumor-bearing animals‡	Survivors§	Average survival time (days±SE)*	Tumor-bearing animals‡	Survivors§	Average survival time (days±SE)*	Tumor-bearing animals‡	Survivors§
Controls	—	—	81.0±12.2	5/5	0/5	100.0± 7.1	5/5	0/5	90.5±7.9	10/10	0/10
Every other day: Days 1–11, 17–27	Daunomycin	2.6	158.4± 9.1**	5/5	0/5	132.8±14.5	5/5	0/5	145.6±9.1**	10/10	0/10
	Daunomycin	3.25	137.8± 7.8**	5/5	0/5	132.2± 9.6	5/5	0/5	135.0±6.3**	10/10	0/10
	Adriamycin	2.0	180.0± 9.0**	2/5	3/5	169.5±11.5*	2/5	3/5	174.7±6.7**	4/10	6/10
	Adriamycin	2.5	200.0¶	1/5	4/5	181.0¶	1/5	4/5	190.5±9.5**	2/10	8/10

*"Student's t" test was calculated by comparing control with treated groups. The probability that the differences between the two groups were not due to chance is indicated at the 95%(*) and 99%(**) levels. "Average survival time" was computed from the time of transplant.

†Long-term survivors are excluded.

‡Transplanted tumor.

§210 days after the beginning of the experiment.

¶Statistical evaluation was impossible because only one observation was available.

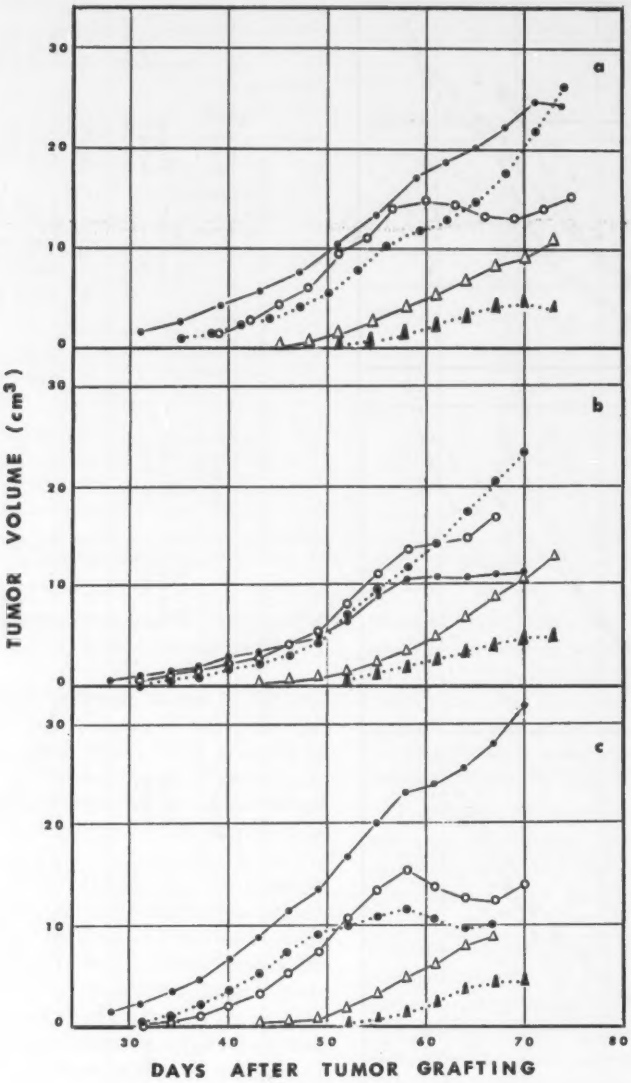

FIGURE 3.—Activity of adriamycin and daunomycin against transplanted mammary carcinoma. Three cycles of daily treatment: from Days 1 to 6, from Days 12 to 14, and from Days 17 to 19 after the transplant. a = Whole population; b = females; c = males. ●———● = Controls. ○———○ = Daunomycin, 2.6 mg/kg/day. ● . . . . . ● = Daunomycin, 3.25 mg/kg/day. △———△ = Adriamycin, 2 mg/kg/day. ▲ . . . . . ▲ = Adriamycin, 2.5 mg/kg/day.

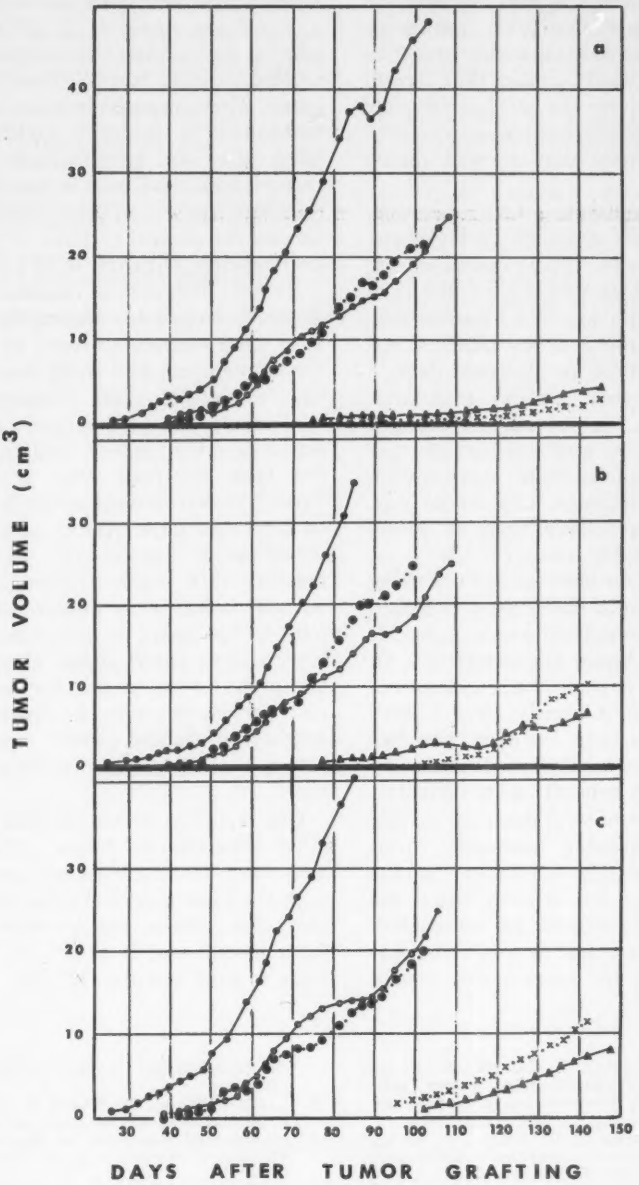

FIGURE 4.—Activity of adriamycin and daunomycin against transplanted mammary carcinoma. Two cycles of treatment every other day, from Days 1 to 11 and from Days 17 to 27 after tumor graft. a = Whole population; b = females; c = males. ●———● = Controls. ○———○ = Daunomycin, 2.6 mg/kg/day. ● . . . . . ● = Daunomycin, 3.25 mg/kg/day. △———△ = Adriamycin, 2 mg/kg/day. × . . . . . × = Adriamycin, 2.5 mg/kg/day.

When administered daily, both adriamycin and daunomycin have significant antitumor effects at the expense of comparable toxicities to the host. Thus, it would appear that significant daunomycin activity can be obtained only with daily injections, although treatment with adriamycin on alternate days is still highly effective.

Toxicity tests carried out on C3H mice (unpublished observations from this laboratory) have shown that, when given continuously, daunomycin at a dose of 3.25 mg/kg/day is as toxic as adriamycin at a dose of 2.5 mg/kg/day, these doses corresponding to the LD10. When the treatment was given on alternate days, a reduction of daunomycin toxicity to a level slightly greater than that of adriamycin was observed. Therefore, we may consider the following doses to be equally toxic: daunomycin, 3.25 mg/kg/day; adriamycin, 2.25 mg/kg/day. These doses are also equally toxic in young Swiss mice after an 8-day treatment (5).

Few experiments have been carried out using this tumor system due to the limited availability of mice bearing the autochthonous tumor. It has been shown that tumor characteristics, including susceptibility to some chemotherapeutic agents, do not vary significantly after a small number of transplants (11). We have therefore extended the study on transplanted mammary carcinoma to the sixth generation transplants.

The effect of treatment with daunomycin and adriamycin on transplanted mammary carcinoma varies considerably according to the schedule of treatment, and in some cases, the sex of the animals. Treatment on consecutive days causes a delay in tumor development that is more evident when the animals are treated with adriamycin, but does not always result in a significant increase of the average survival time of the animals over control values; in the treated animals, increased metastasis was often noted. These negative results can be explained tentatively by the high toxicity of the drugs when they are administered daily. The increased metastasis can be attributed to the immunodepressive activity which has been described for several systems of daunomycin and adriamycin treatment (5,12,13).

Recent distribution studies with tritiated daunomycin and adriamycin have shown that a 50% drug excretion occurs in about 24 hours for daunomycin and in 32 hours for adriamycin; moreover, after repeated daily doses, adriamycin is accumulated in the various organs to a high extent, while such an effect has not been observed with daunomycin (14). Therefore, a treatment schedule using injections on alternate days over a long period of time would seem appropriate, particularly when treating this tumor system with adriamycin without inducing an intolerable degree of toxicity to the host. In fact, when the two antibiotics were administered every other day, in two cycles of six treatments each with a 5-day rest period, not only a significant delay and decrease of tumor growth was observed, but also a marked increase in the average survival time.

Our report is consistent with observations in other experimental tumor systems of greater antitumor activity with adriamycin (3–5) and with the preliminary clinical trials, which suggest that adriamycin is more effective than daunomycin, and is active on a broader spectrum of solid tumors (15–17).

REFERENCES

1. Arcamone, F., Cassinelli, G., Fantini, C., et al. Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from *Streptomyces peucetius* var. *caesius*. Biotechnol Bioeng 11:1101–1110, 1969.
2. Arcamone, F., Franceschi, G., Penco, S., et al. Adriamycin (14-hydroxydaunomycin) a novel antitumor antibiotic. Tetrahedron Lett 13:1007–1010, 1969.
3. Di Marco, A., Gaetani, M., and Scarpinato, B. Adriamycin (NSC-123,127): a new antibiotic with antitumor activity. Cancer Chemother Rep 53:33–37, 1969.
4. Sandberg, J. S., Howsden, F. L., Di Marco, A., et al. Comparison of the antileukemic effect in mice of adriamycin (NSC-123127) with daunomycin (NSC-82151). Cancer Chemother Rep 54:1–7, 1970.
5. Casazza, A. M., Di Marco, A., and Di Cuonzo, G. Interference of daunomycin and adriamycin on the growth and regression of Murine Sarcoma Virus (Moloney) tumors in mice. Cancer Res 31:1971–1976, 1971.
6. Di Marco, A., Zunino, F., Silvestrini, R., et al. Interaction of some daunomycin derivatives with deoxyribonucleic acid and their biological activity. Biochem Pharmacol 20:1323–1328, 1971.
7. Silvestrini, R., Gambarucci, C., and Dasdia, T. Attività biologica dell'adriamicina in vitro. Tumori 56:137–148, 1970.
8. Chou, Y. Statistical Analysis: With Business and

Economic Applications. New York, Holt, Rinehart and Winston, Inc., 1969, p 556.
9. MIHICH, E., and GELZER, J. Effects of 4,4'-diacetyldiphenyl-urea-bis(guanylhydrazone) on a spectrum of mouse and rat tumors. Cancer Res 28:553–558, 1968.
10. DUKOR, P., SALVIN, S. B., DIETRICH, F. M., ET AL. Effect of reserpine on immune reactions and tumor growth. Eur J Cancer 2:253–261, 1966.
11. SCHOLLER, J., PHILIPS, F. S., STERNBERG, S. S., ET AL. A comparative study of chemotherapeutic agents in spontaneous mammary adenocarcinomas of mice and in transplants of recent origin. Cancer 9:240–251, 1956.
12. DADDI, G., JR., INTINI, C., ISETTA, A. M., ET AL. Daunomycin and skin allografts survival in mice. Experientia 26:910–911, 1970.
13. ISETTA, A. M., INTINI, C., and SOLDATI, M. On the immunodepressive action of adriamycin. Experientia 27:202–204, 1971.
14. DI FRONZO, G., GAMBETTA, R. A., and LENAZ, L. Distribution and metabolism of adriamycin in mice. Comparison with daunomycin. Rev Eur Etud Clin Biol [formerly Rev Franc Etud Clin Biol] 16: 572–576, 1971.
15. BONADONNA, G., MONFARDINI, S., DE LENA, M., ET AL. Phase I and preliminary phase II evaluation of adriamycin (NSC-123127). Cancer Res 30:2572–2582, 1970.
16. TAN, C., WOLLER, N., KING, O., ET AL. Adriamycin, a new antibiotic, in treatment of childhood leukemia and other malignant neoplasms. Proc Am Assoc Cancer Res 11:79, 1970.
17. BONADONNA, G., DE LENA, M., and BERETTA, G. Letter to the Editor: Preliminary clinical screening with adriamycin in lung cancer. Eur J Cancer 7:365–367, 1971.

Antitumor Effects of the Antispermatogenic Agent, 2,3-Dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone (NSC-145669) [1, 2, 3]

Gary L. Neil, L. H. Li, Harold H. Buskirk, and Thomas E. Moxley [4,5]

SUMMARY

The antitumor activities of 2,3-dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone (NSC-145669), an antispermatogenic agent, and a number of related quinazolinone derivatives have been studied. NSC-145669 inhibits significantly the growth of L1210 cells in culture; when administered intraperitoneally (ip), subcutaneously (sc), or orally to ip inoculated L1210 leukemic mice, NSC-145669 significantly increases lifespan. (Increases in lifespan in excess of 100% were achieved.) NSC-145669 administered ip or sc is active also against intravenously inoculated L1210 leukemia. When administered ip it is active against P388 leukemia in mice. Daily ip treatment of normal mice with NSC-145669 results in noticeable decreases in thymus weights with little effect on the spleen. The other quinazolinone derivatives studied were much less growth inhibitory in vitro and none significantly increased the lifespan of L1210 leukemic mice. DNA synthesis in L1210 cells in culture is more inhibited by NSC-145669 than either RNA or protein synthesis.

[Cancer Chemother Rep 56:163–173, 1972]

The effects of antitumor agents on proliferating tissues other than tumor cells are well known (1,2). Many of these agents exert toxic effects on hematopoietic tissue, resulting in, for example, immunosuppressive activity (2). Embryonic tissue is often susceptible, and some antitumor agents are teratogens (1,3,4) or abortifacients (4,5) or both. Gastrointestinal toxicity (1) and alopecia (1,3) caused by many antitumor agents are manifestations of their cytotoxic effects on other rapidly proliferating normal tissues.

The effects of antitumor agents on another rapidly proliferating normal cell population, that involved in spermatogenesis, have recently received attention (6,7). Spermatogenesis has been shown to be sensitive to several cytotoxic agents used in the treatment of neoplastic disease: diesters of methanesulfonic acid (eg, busulfan) (8); the aziridine, triethylenemelamine (8); and cyclophosphamide (7) have antispermatogenic properties.[6]

[1] Received July 22, 1971; revised Oct 29, 1971; accepted Nov 2, 1971.

[2] Supported in part by contract PH43-68-1023 from Chemotherapy, National Cancer Institute.

[3] 2,3-Dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone: U-29,409.

[4] Cancer Research, The Upjohn Company, Kalamazoo, Mich.

[5] The excellent technical assistance of Miss Sandra L. Kuentzel, Miss Lynda G. Scheidt, Mrs. Elma J. Olin, and Mrs. Joan W. Culp is gratefully acknowledged. We thank Drs. David G. Martin and Gilbert A. Youngdale and the Biological Screening Office (The Upjohn Company) for providing the agents used in this study. We wish to thank Drs. R. J. Ericsson and E. M. Glenn for helpful discussions and for making their data available prior to publication. The editorial assistance of Mrs. Elizabeth L. Clark is also acknowledged.

[6] Busulfan: NSC-750; 1,4-butanediol, dimethanesulfonate; Myleran.

Triethylenemelamine: NSC-9706; *s*-triazine, 2,4,6-tris(1-aziridinyl)-; TEM; tretamine.

Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

Because of these effects and the probable similarities between the inhibition of the proliferation of a tumor cell population and the arrest of spermatogenesis, we have tested a number of male antispermatogenic agents for antitumor activity in L1210 and P388 leukemic mice. One of these agents, 2,3-dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone (NSC-145669), reported by Ericsson (9) to possess reversible male antifertility activity in the rat, showed considerable antitumor activity. This activity and that of some other quinazolinone derivatives are reported here.

MATERIALS AND METHODS

The structures of NSC-145669 and the other agents studied are shown in figure 1.[7]

Growth-Inhibitory Studies

The effects of the agents on growth of L1210 mouse leukemia cells in culture were determined by the method of Buskirk (10). L1210 cells were maintained in RPMI-1634 medium (Grand Island Biological Company, Grand Island, NY) supplemented with sodium bicarbonate (0.075% w/v), penicillin (0.1 mg/ml), and streptomycin (0.05 mg/ml).

To insure solubility at the levels used to determine growth inhibition (<10μg/ml medium), stock 1-mg/ml aqueous solutions (or suspensions) contained 2% ethanol (v/v) (for sterilization) and 10% dimethylsulfoxide (v/v). The final concentrations of solvents in the tissue culture tubes never exceeded 0.02% v/v (ethanol) or 0.1% v/v (dimethylsulfoxide). These solvent levels did not appreciably affect cell growth. Growth after 3 days' incubation at 37 C was measured by electronic particle counting (Coulter Counter, Model FN, Coulter Electronics, Hialeah, Fla) and percent inhibition of growth (compared to control samples) was calculated for serial agent dilutions by the method of Smith et al (11).

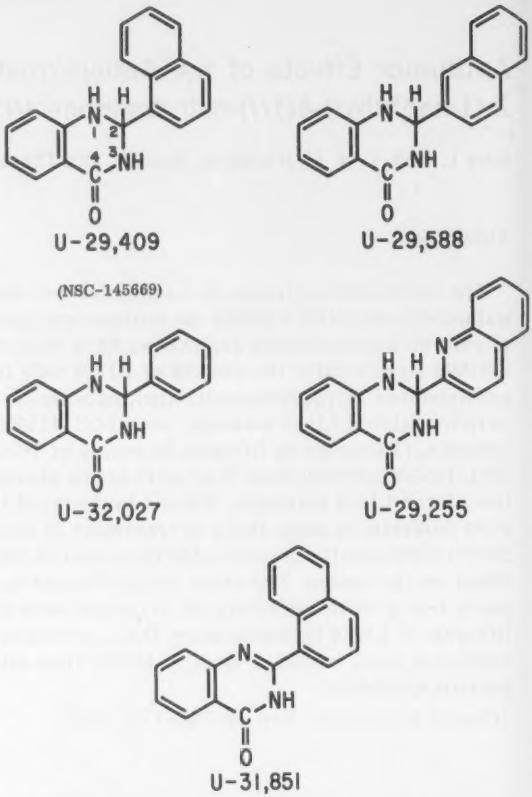

FIGURE 1.—2,3-Dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone (NSC-145669) and other quinazolinone derivatives.

Antitumor Evaluation

The methods used in studying the effects of agents on survival of L1210 and P388 leukemic mice were similar to those described for L1210 leukemia by Drug Research and Development (formerly the Cancer Chemotherapy National Service Center), National Cancer Institute (12). Parent lines were obtained from I. Wodinsky, Arthur D. Little, Inc., Cambridge, Massachusetts (L1210), and E. Wayne Artis, Microbiological Associates, Inc., Bethesda, Maryland (P388), and were maintained by weekly passage (intraperitoneal [ip] inoculation) of ascites cells in female DBA/2 mice

[7] NSC-145669 (U-29,409): Melting point (mp) = 187-189 C; molecular weight (mol wt) = 274.31. U-29,588: 2,3-dihydro-2-(2-naphthyl)-4(1*H*)-quinazolinone; mp = 220-222 C; mol wt = 274.31. U-32,027: 2,3-dihydro-2-phenyl-4(1*H*)-quinazolinone; mp = 223-226 C; mol wt = 224.25. U-29,255: 2,3-dihydro-2-(2-quinolyl)-4(1*H*)-quinazolinone; mp = >300 C; mol wt = 275.30. U-31,851: 2-(1-naphthyl)-4(3*H*)-quinazolinone; mp = 289-290 C; mol wt = 272.30.

(Jackson Memorial Laboratories, Bar Harbor, Me). Therapeutic tests were conducted in female[8] BDF_1 mice (C57BL/6 × DBA/2) from the same source, weighing 19 ± 2 g. Mice were inoculated ip (or intravenously [iv]) with L1210 cells (10^5 per mouse) on Day 0 and therapy was started 24 hours later (see tables and figures for treatment routes and schedules).

Agents were administered ip, subcutaneously (sc), or by oral gavage to mice as suspensions in vehicle 122 (0.25% aqueous methylcellulose; 0.2 ml volume). Animals were weighed on Day 0 or Day 1 and again on Day 5, and average weight changes (g/mouse) were determined. Percent increases in lifespan (% ILS) were calculated from median survival times and a 25% ILS was considered to indicate significant antitumor activity. Forty-five-day survivors were defined as "cures."

The effects of NSC-145669 on organ weights of female BDF_1 mice were determined after 5 days of treatment (100 mg/kg/day, ip). A control group of animals received only vehicle 122.

[8] Preliminary studies in both male and female BDF_1 mice (L1210 leukemia) indicated that there was no significant sex difference with regard to the antitumor effects of NSC-145669.

TABLE 1.—Inhibition of growth of L1210 cells in culture by quinazolinone derivatives

Agent	ID50* (μg/ml)	ID90* (μg/ml)
NSC-145669 (U-29, 409)	0.022	0.036
U-32,027	1.7	2.5
U-29,255	3.7	>10†
U-29,588	10†	>10†
U-31,851	>10†	>10†

* ID50 and ID90 values are the concentrations required to inhibit growth of L1210 cells in culture (over a 3-day period) 50% and 90%, respectively.
† Highest concentration used was 10 μg/ml.

Each group consisted of eight mice. On Day 7, individual spleen and thymus weights were obtained and compared to total body weight (table 3).

Effects of NSC-145669 on Macromolecular Synthesis of L1210 Cells in Culture

The method used to study the effects of NSC-145669 on the incorporation of ^{3}H-TdR, ^{3}H-UR, and ^{14}C-leucine[9] into the DNA, RNA, and pro-

[9] ^{3}H-TdR; thymidine-methyl-^{3}H. ^{3}H-UR; uridine-^{3}H (g.l.). ^{14}C-Leucine, DL-leucine-1-^{14}C. All labeled precursors were obtained from the New England Nuclear Corporation, Boston, Mass.

TABLE 2.—Antitumor effects of quinazolinone derivatives in L1210 leukemic mice*

Agent	Dose† (mg/kg/day, Days 1–5)	Median survival time (days)	% ILS‡	Weight change, Days 1–5 (g/mouse)
Control	—	8.5	—	+1.5
NSC-145669 (U-29,409)	50	11.5	35	−1.3
	75	14.0	65	−1.9
	100	14.5	71	−2.7
U-32,027	50	8.0	0	+0.1
	100	8.5	0	−0.2
	150	9.0	6	−0.5
U-29,255	50	8.0	0	+0.4
	100	8.0	0	−0.9
	150	8.0	0	−3.0
U-29,588	50	8.5	0	0
	100	8.0	0	−0.4
	150	8.0	0	−0.2
U-31,851	50	8.0	0	−0.5
	100	8.0	0	+1.5
	150	8.0	0	−1.3

*Mice received ip inoculations of 1×10^5 L1210 cells/mouse on Day 0.
†Treatment (ip) was started on Day 1 and continued daily for 5 days.
‡% ILS was calculated from median survival times of control and treated groups (8 mice/group).

tein, respectively, of L1210 cells growing in culture has been described (13). Labeled precursors were added immediately after addition of NSC-145669 to L1210 cells (5×10^5 cells/ml medium). Reaction mixtures contained dimethylsulfoxide (0.8%, v/v). Aliquots were removed at 1-hour intervals over the next 3 hours and specific incorporations (cpm/μg macromolecule) were determined. Incorporation rates (cpm/μg macromolecule/hr) and Pearson's correlation coefficients (r) were calculated by linear regression techniques.

Percent inhibition due to NSC-145669 was calculated by comparing rates of incorporation to those observed in a solvent control sample

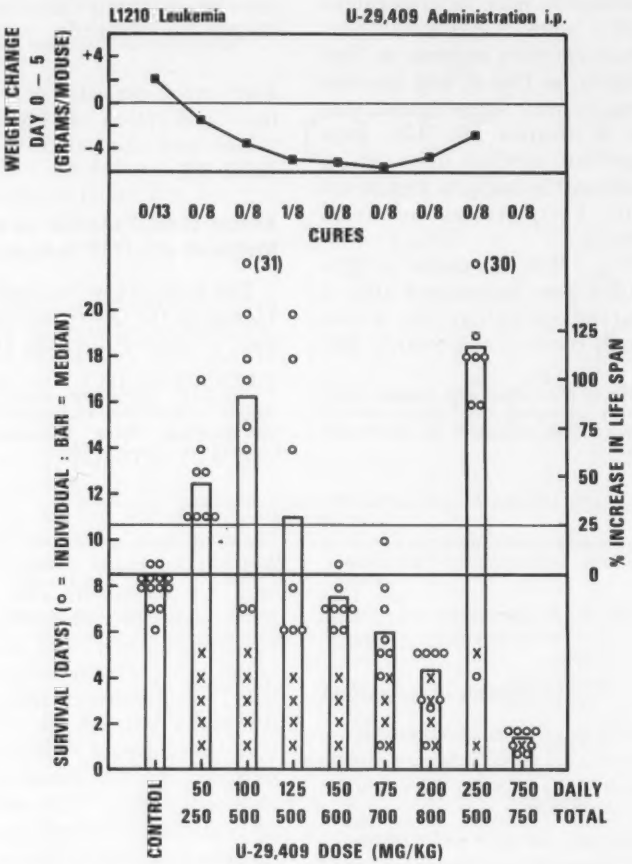

FIGURE 2.—Ip treatment of L1210 leukemic mice with NSC-145669 (U-29,409). Mice received inoculations of 10^5 L1210 cells/mouse on Day 0. Days of treatment are indicated by ×. For clarity, individual survival times (○) are shown only to the nearest day. Median survival times (bars) were calculated from survival data to the nearest half-day. Average weight changes (g/mouse) (■) were based on weights on Days 5 and 0. Both daily and total doses are shown. "Cures" represent fractions of animals treated that survived for 45 days. Percent ILS was calculated from median survival times of treated and control (untreated) groups.

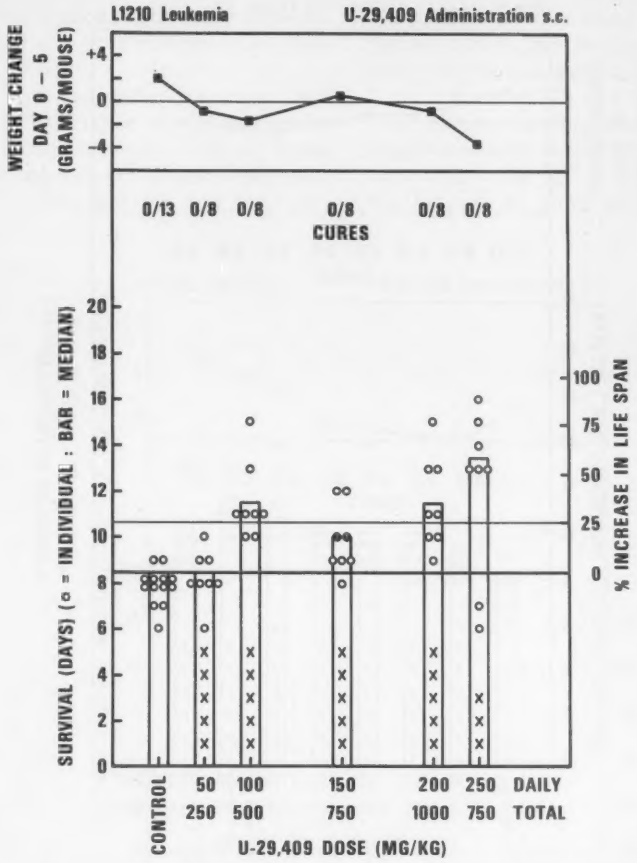

FIGURE 3.—Sc treatment of L1210 leukemic mice with NSC-145669 (U-29,409). Mice received inoculations of 10^5 L1210 cells/mouse on Day 0. Days of treatment are indicated by ×. For clarity, individual survival times (○) are shown only to the nearest day. Median survival times (bars) were calculated from survival data to the nearest half-day. Average weight changes (g/mouse) (■) were based on weights on Days 5 and 0. Both daily and total doses are shown. "Cures" represent fractions of animals treated that survived for 45 days. Percent ILS was calculated from median survival times of treated and control (untreated) groups.

(containing dimethylsulfoxide). A control without dimethylsulfoxide was also included.

RESULTS

The effects of five quinazolinone derivatives on the growth of L1210 mouse leukemia cells in culture are shown in table 1. NSC-145669 was significantly growth inhibitory, having ID50 and ID90 values of 0.022 and 0.036 μg/ml, respectively. It was about 100 times more growth inhibitory than any of the other derivatives.

The effects of the five agents administered ip daily (Days 1–5) on the survival of L1210 leukemic mice are shown in table 2. Only NSC-145669 showed activity. Considerable weight

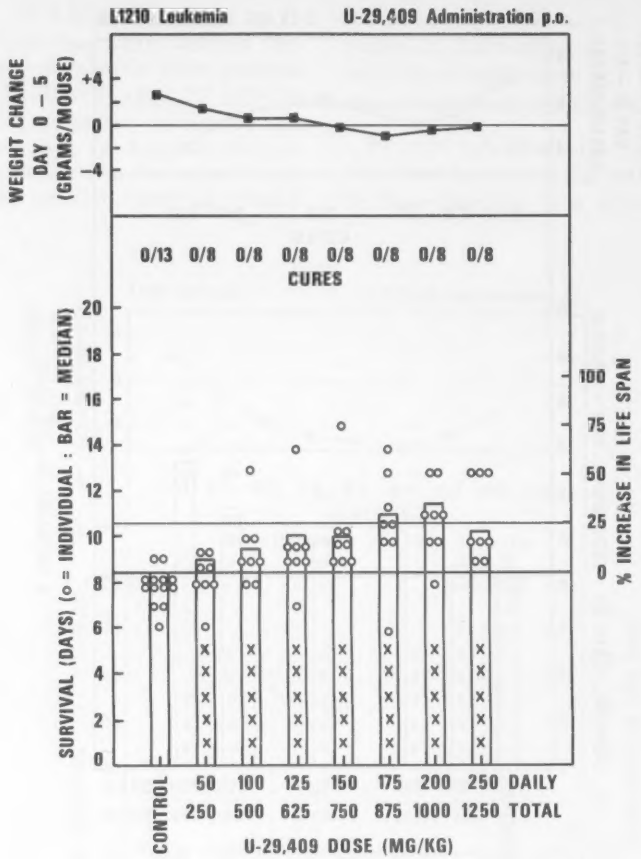

FIGURE 4.—Oral treatment of L1210 leukemic mice with NSC-145669 (U-29,409). Mice received inoculations of 10^5 L1210 cells/mouse on Day 0. Days of treatment are indicated by ×. For clarity, individual survival times (○) are shown only to the nearest day. Median survival times (bars) were calculated from survival data to the nearest half-day. Average weight changes (g/mouse) (■) were based on weights on Days 5 and 0. Both daily and total doses are shown. "Cures" represent fractions of animals treated that survived for 45 days. Percent ILS was calculated from median survival times of treated and control (untreated) groups.

loss (1.3–2.7 g/mouse, Days 1–5) was observed also. The other derivatives had some toxic manifestations (as indicated by weight loss), but no antitumor effects.

Dose responses after ip, sc, and oral administration of NSC-145669 to L1210 leukemic mice and after ip administration to P388 leukemic mice are shown in figures 2–5. The optimum daily dose for ip administration (Days 1–5) of NSC-145669 to L1210 leukemic mice was 100 mg/kg/day (88% ILS) (fig 2). Higher doses resulted in considerable lethal toxicity. Two ip doses of 250 mg/kg administered 4 days apart (Days 1 and 5) were slightly more effective (118% ILS) than five daily doses of 100 mg/kg. Results quite similar to those noted

with L1210 leukemic mice (fig 2) were observed when NSC-145669 was administered ip to P388 leukemic mice (fig 5).

NSC-145669 was less effective (and less toxic) when administered sc (fig 3) and orally (fig 4) to L1210 leukemic mice. With sc doses of 100 and 200 mg/kg/day (Days 1–5), significant (> 25% ILS) activity was observed. Three daily 250-mg/kg sc doses of NSC-145669 yielded a 59% ILS, but toxicity (manifested by early deaths and weight loss) also increased.

Oral doses of 175 and 200 mg/kg/day (Days 1–5) resulted in significant life prolongation of L1210 leukemic mice (29% and 35% ILS, respectively) (fig 4). A daily oral dose of 250 mg/kg was no more effective than the lower

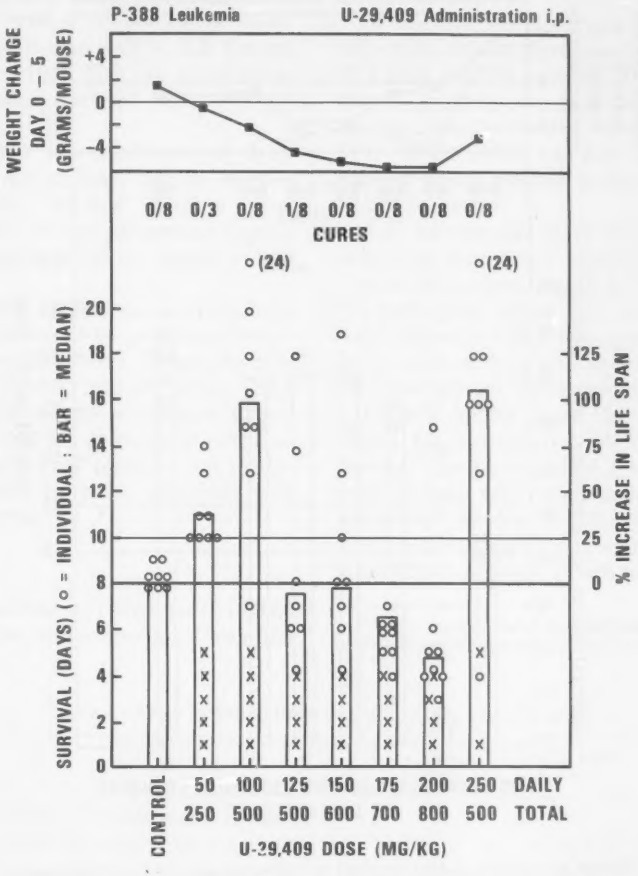

FIGURE 5.—Ip treatment of P388 leukemic mice with NSC-145669 (U-29,409). Mice received inoculations of 10^5 P388 cells/mouse on Day 0. Days of treatment are indicated by ×. For clarity, individual survival times (○) are shown only to the nearest day. Median survival times (bars) were calculated from survival data to the nearest half-day. Average weight changes (g/mouse) (■) were based on weights on Days 5 and 0. Both daily and total doses are shown. "Cures" represent fractions of animals treated that survived for 45 days. Percent ILS was calculated from median survival times of treated and control (untreated) groups.

doses. Little weight loss was evident at this dose level however, and higher doses may be possible.

When NSC-145669 was administered as a single 250-mg kg ip dose (Day 1) to L1210 leukemic mice, a 35% ILS was observed. Doses of 350 mg kg and higher were lethal. The single dose LD50 (ip) appears to be between 250 and 300 mg/kg.

The effects of dose interval between two 250-mg/kg ip doses of NSC-145669 on the survival of L1210 leukemic mice are shown in figure 6. Two 250-mg/kg doses administered simultaneously are, of course, lethal. Dose intervals

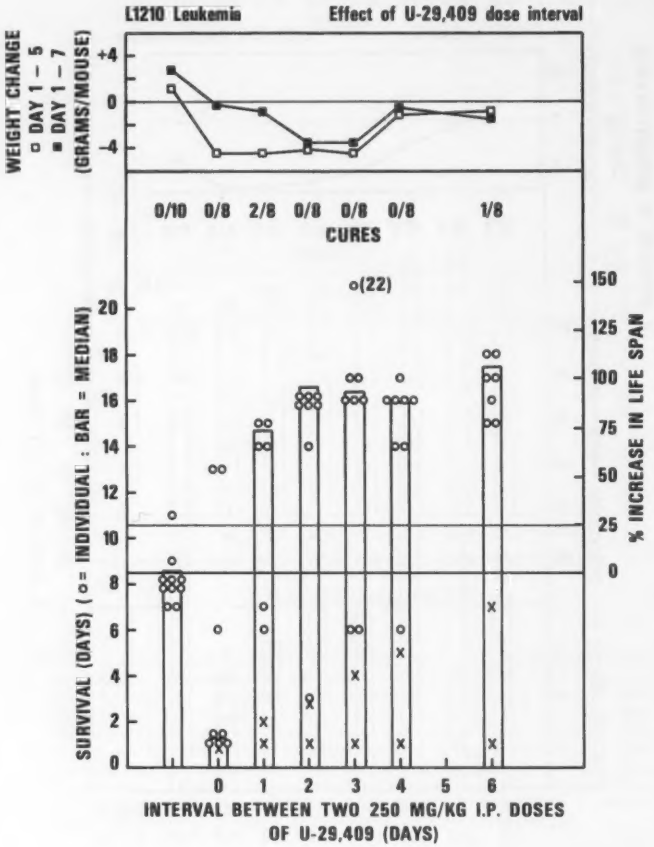

FIGURE 6.—Effect of dose interval in the treatment of L1210 leukemic mice with NSC-145669. Mice received inoculations of 10^5 L1210 cells/mouse on Day 0. Two 250-mg/kg ip doses of NSC-145669 were administered at different intervals, beginning on Day 1. Days of treatment are indicated by ×. For clarity, individual survival times (○) are shown only to the nearest day. Median survival times (bars) were calculated from survival data to the nearest half-day. Average weight changes (g/mouse) (□,■) were based on weights on Days 5 (or 7) and 1. "Cures" represent fractions of animals treated that survived for 45 days. Percent ILS was calculated from median survival times of treated and control (untreated) groups.

of 1, 2, 3, 4, or 6 days yielded approximately the same survival increase (about 75%–105%). The shorter intervals resulted in somewhat more toxicity (weight loss and early deaths). On the other hand, two of eight mice were cured (45-day survivors) when the interval was only 1 day.

NSC-145669 administered ip or sc was effective against iv inoculated L1210 leukemia also. Daily 100-mg/kg doses (Days 1–5) given to mice inoculated iv with 1×10^5 L1210 cells/mouse on Day 0 resulted in a 78% ILS (ip administration) and a 72% ILS (sc administration). Control median survival time was 7.0 days.

When NSC-145669 was administered daily for 5 days (100 mg/kg/day, ip) to normal female BDF_1 mice, thymus weights (and thymus-body weight ratios) decreased significantly (table 3). Little effect on spleen weight was observed.

The effects of NSC-145669 on macromolecular synthesis (as measured by precursor incorporation) are shown in table 4. When the concentration of NSC-145669 was 20 μg/ml, DNA synthesis was inhibited to a greater extent (56%) than RNA (10%) or protein synthesis (26%). Dimethylsulfoxide (used to aid in dissolving NSC-145669) by itself inhibited only RNA synthesis (37%).

DISCUSSION

Ericsson has reported (9) that NSC-145669 causes the exfoliation and migration (from the testes to excurrent ducts) of seminiferous epithelium cells, resulting in prolonged infertility in male rats. Also, NSC-145669 has been shown to inhibit spermatogenesis in mice (R. J. Ericsson, unpublished results). Glenn et al (15) have shown that NSC-145669 inhibits the development of adjuvant-induced polyarthritis and allergic encephalomyelitis in rats, but has no significant inhibitory effect in rats with established arthritis.

Of the five quinazolinone derivatives tested, only NSC-145669 significantly inhibited growth of L1210 cells in culture. The ID50 value observed is similar to that (approximately 0.01 μg/ml) observed with cytosine arabinoside[10] (14). NSC-145669 was also the only compound studied which showed activity in vivo against L1210 leukemia.

NSC-145669 was more effective when administered ip than when administered sc or orally to mice inoculated ip with L1210 leukemia. That the antitumor effect was not due entirely to local contact toxicity was apparent when it was found that both ip and sc administration were effective against the iv inoculated disease.

Little is known about the structural requirements for antitumor activity of the quinazolinones. The limited data presented here, however, at least offer some suggestions. The "unsaturated" analog of NSC-145669 (U-31,851) was devoid of activity in vitro and in vivo. The 2-naphthyl analog (U-29,588) was at least

[10] Cytosine arabinoside: NSC-63878; cytosine, 1-β-D-arabinofuranosyl, monohydrochloride; ara-C.

TABLE 3.—Effect of NSC–145669 on organ weights of BDF_1 mice*

	Vehicle control	NSC–145669 treated
Total body weight, Day 0 (g/mouse)	17.7	17.2
Total body weight, Day 7 (g/mouse)	18.4±1.4	17.0±1.6
Spleen weight, Day 7 (mg/mouse)	75±11	59±18
Thymus weight, Day 7 (mg/mouse)	62±9	23±7
Spleen-body weight ratio ($\times 10^3$)	4.0±0.5	3.4±0.8
Thymus-body weight ratio ($\times 10^3$)	3.4±0.6	1.3±0.3

*Female BDF_1 mice (8/group) were treated ip daily (Days 0–5) with vehicle 122 (control) or NSC–145669 (100 mg/kg/day). Animals were weighed as a group on Day 0. On Day 7, animals were weighed individually and killed, and individual thymus and spleen weights were determined. Individual organ-body weight ratios were calculated. Means with standard deviations are shown.

TABLE 4.—Effects of NSC-145669 on macromolecular synthesis by L1210 cells in culture

NSC-145669 concentration (μg/ml)	Dimethylsulfoxide concentration (% v/v)	DNA* Incorporation rate	DNA* r	DNA* Inhibition (%)	RNA* Incorporation rate	RNA* r	RNA* Inhibition (%)	Protein* Incorporation rate	Protein* r	Protein* Inhibition (%)
0	0	450.0	0.9996	(3)	1180.0	0.9948	(37)	13.7	0.9989	8
0	0.8	436.0	1.0000	0	860.0	0.9928	0	14.9†	—†	0
4	0.8	303.0	0.9996	31	910.0	0.9827	(6)	12.6	0.9987	15
20	0.8	193.0	0.9974	56	770.0	0.9851	10	11.0	0.9986	26

*Precursors were: DNA, ^{3}H-TdR (1.9 μc/3.4 μg/ml); RNA, ^{3}H-UR (1.9 μc/3.4 μg/ml); and protein, ^{14}C-leucine (0.2μc/1.95 mg/ml). Cell concentration was 5×10^5/ml medium. Incorporation rates (cpm/μg macromolecule/hr) and Pearson's correlation coefficients (r) were calculated by linear regression techniques. Percent inhibitions were calculated by comparison of rates with incorporation rates of a control sample containing only dimethylsulfoxide. Numbers in parentheses represent stimulation.

†Rate determined from only 2 data points. Pearson's correlation coefficient was not calculated.

1000-fold less growth inhibitory in culture and showed no antitumor activity in vivo. The 2-quinolyl derivative (U-29,255) was also essentially inactive in both systems. The only agent which showed significant activity in vitro was U-32,027, the phenyl analog. Yale and Kalkstein (16) found that this agent significantly inhibited growth of Earle's L cells in suspension. Some 5- and 6-substituted derivatives of U-32,027 have been reported to have antitumor activity in vivo against the rat Walker 256 carcinoma (17). Our studies suggest that for activity against L1210 leukemia (in vitro and in vivo), the 1-naphthyl group and saturation of the pyrimidine ring appear to be required. Further structure-activity studies are in progress.

Although DNA synthesis (in L1210 cells in culture) is inhibited to a greater extent than RNA or protein synthesis (table 4), the concentration required to inhibit the former process is considerably greater (about 1000 times) than that required to inhibit growth by a comparable amount (table 1). Thus, it is impossible to state that DNA synthesis inhibition is necessarily responsible for the cytotoxic activity of NSC-145669. Attempts are presently being made to determine unequivocally the primary biochemical locus of action of this agent.

The antileukemic activity of NSC-145669 reported here, as well as its unique effects on the male reproductive system (9), suggests that further studies of the potential use of this agent in the treatment of neoplastic disease are warranted.

REFERENCES

1. FREI, E., III. Effect of cancer chemotherapeutic agents on normal tissues in man. Fed Proc 26:918–922, 1967.
2. HAINES, R. F., JOHNSON, A. G., and PETERING, H. G. Variable influences of antitumor drugs and progestational agents on immune responses in rodents. Fed Proc 26:934–942, 1967.
3. KARNOFSKY, D. A. Late effects of immunosuppressive anticancer drugs. Fed Proc 26:925–932, 1967.
4. HERTZ, R., LEWIS, J., JR., and LIPSETT, M. B. Five years' experience with the chemotherapy of metastatic choriocarcinoma and related trophoblastic tumors in women. Am J Obstet Gynecol 82:631–640, 1961.
5. SVATA, M., RASKA, K., JR., and SORM, F. Interruption of pregnancy by 5-azacytidine. Experientia 22:53, 1966.
6. LEE, I. P., and DIXON, R. L. Drug effects on spermatogenesis studied by velocity sedimentation cell separation. (Abstr) Toxicol Appl Pharmacol 19:370, 1971.
7. JACKSON, H. Antispermatogenic agents. Br Med Bull 26:79–86, 1970.
8. JACKSON, H. The effects of alkylating agents on fertility. Br Med Bull 20:107–114, 1964.
9. ERICSSON, R. J. Antispermatogenic properties of 2,3,-dihydro-2-(1-naphthyl)-4(1*H*)-quinazolinone (U-29,409). Proc Soc Exp Biol Med 137:532–535, 1971.
10. BUSKIRK, H. H. Assay of cytotoxic agents with L1210 cells. Proc Tissue Culture Assoc 20:23, 1969.
11. SMITH, C. G., LUMMIS, W. L., and GRADY, J. E. An improved tissue culture assay. I. Methodology and cytotoxicity of anti-tumor agents. Cancer Res 19:843–846, 1959.
12. Protocols for screening chemical agents and natural products against animal tumors and other biological systems—Cancer Chemotherapy National Service Center. Cancer Chemother Rep 25:1–66, 1962.
13. LI, L. H., OLIN, E. J., BUSKIRK, H. H., ET AL. Cytotoxicity and mode of action of 5-azacytidine

on L1210 leukemia. Cancer Res 30:2760–2769, 1970.
14. NEIL, G. L., BUSKIRK, H. H., MOXLEY, T. E., ET AL. Biochemical and pharmacologic studies with 1-β-D-arabinofuranosylcytosine 5′-adamantoate (NSC-117614), a depot form of cytarabine. Biochem Pharmacol 20:3295–3308, 1971.
15. GLENN, E. M., LYSTER, S. C., and ROHLOFF, N. A. Antiarthritic effects of the antispermatogenic agent 2,3-dihydro-(1-naphthyl)-4-(1*H*)-quinazolinone (U-29,409). Proc Soc Exp Biol Med 138:244–248, 1971.
16. YALE, H. L., and KALKSTEIN, M. Substituted 2,3-dihydro-4(1*H*)-quinazolinones. A new class of inhibitors of cell multiplication. J Med Chem 10:334–336, 1967.
17. YALE, H. L. 2,3-Dihydro-5 and 6-substituted-2-phenyl-4(1*H*)-quinazolinones. US Pat 3,455,920, July 15, 1969.

Combination Chemotherapy in Isolation Perfusion: Use of Mitotic Inhibitors in Pretreatment of V × 2 Carcinoma Undergoing Isolation Perfusion With Alkylating Agents[1, 2]

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SUMMARY

This experiment was designed to investigate whether systemic pretreatment with metaphase-arresting agents would enhance the effectiveness of cancer chemotherapy by isolation perfusion. Rabbit V × 2 carcinoma was transplanted to the hind limbs of New Zealand white rabbits and the limbs were perfused for 40–60 minutes with nitrogen mustard or L-phenylalanine mustard (melphalan) after systemic pretreatment with the mitotic inhibitors, demecolcine (Colcemid) or vinblastine. Demecolcine pretreatment improved the effect of nitrogen mustard perfusion by extending the survival time, though no "cure" was obtained by this combination. It also improved the therapeutic effect of melphalan perfusion in V × 2 carcinoma by extending the survival time, though it failed to modify the curability of melphalan perfusion, which was as high as 47.4% by itself. Pretreatment with vinblastine was more effective than pretreatment with demecolcine, increasing the "cures" from the melphalan perfusion to 61.6% and extending the survival time of the remaining animals that eventually died of the disease. On the whole, melphalan perfusion was very effective in our strain of V × 2 carcinoma, and the combination of vinblastine pretreatment and melphalan perfusion was the best regimen in this investigation, suggesting that a pretreatment with some mitotic inhibitor is a feasible way to enhance the effects of perfusion chemotherapy for cancer.

[Cancer Chemother Rep 56:175–181, 1972]

Cancer chemotherapy by regional perfusion was first reported in 1958 by Oscar Creech, Jr. and his co-workers (1). Since that time, perfusion chemotherapy has been performed at many institutions throughout the world, and techniques have been refined and indications have become more clarified. At present, the main indication for this treatment is as an adjunct to conventional excision of certain localized malignant tumors. Another indication is for palliation in more advanced cases, resulting in temporary regression of the tumor and relief of pain (2–4). Along with these clinical

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applications, many investigations have been carried out to find the optimum conditions for perfusion. The optimum conditions of the perfusates were investigated extensively, including the optimal pH (5), CO_2 content (6), O_2 content (7–9), flow rate and pressure (10), and temperature (10, 11). The use of histamine and vasodilators has also been suggested to attain an increased cell permeability or an increased drug flow (12). Another means of improving the effectiveness of cancer chemotherapy is selection of the drug (13) or combination of several drugs (14–18) most suitable for the tumor being perfused. Combination chemotherapy often has a greater effect than single agent therapy on neoplastic disease, but its effectiveness is generally limited to systemic diseases such as leukemia, Hodgkin's disease, lymphosarcoma, and chorionepithelioma. In localized malignant tumors, however, especially in tumors of the extremities, perfusion chemotherapy is more effective than systemic chemotherapy, even when a combined systemic regimen is used. The reason for this superiority of the isolation perfusion is apparently its ability to deliver much higher concentrations of anticancer agents to the tumor with decreased systemic toxicity. This technique, however, can only be repeated once or twice with a maximum perfusion time of 2 hours or less. Thus, although the tumor can be exposed to a relatively high concentration of cancer chemotherapeutic agent, a 100% destruction of cancer cells may not be expected during this short procedure without damaging surrounding normal tissues. Accordingly, an experiment to achieve a uniform synchrony of tumor cell population was attempted by previous exposure to a metaphase-arresting agent that would then allow perfusion with anticancer agents to occur at a time when the cells are most sensitive to the drug or when they have an increased growth fraction (19–22).

MATERIALS AND METHODS

About 100 female New Zealand white rabbits weighing 3.0–4.0 kg each were used in the present experiment. The V × 2 carcinoma[8] was ground with a Potter glass homogenizer to make a 10% suspension in isotonic saline, and 0.3 ml of this homogeneous suspension was injected into the calf muscle of the left hind limb. Thirteen to fourteen days after the transplantation, the tumor measured 1.3–1.5 cm in diameter, at which time the isolation perfusion was carried out. The small extracorporeal circulatory system reported by Mori et al (23) was used. The flow rate was 3–4 ml/minute and a constant flow of an oxygen mixture (98% O_2 and 2% CO_2) was used to oxygenize the perfusing blood. The temperature of the perfusate was maintained at approximately 38 C by warming with a heating wire wound around the oxygenator. The diameter of the tumor was determined in three dimensions by external palpation combined with measurement with calipers at 2- or 3-day intervals, and the mean diameter was calculated according to the formula[9] suggested by Larionov (24).

Two metaphase-arresting agents, demecolcine (Colcemid) and vinblastine, were used for the pretreatment, and two alkylating agents, nitrogen mustard and L-phenylalanine mustard (melphalan), were used for the perfusion.[10] The pretreatment was started 28 hours before the perfusion, with demecolcine or vinblastine given systemically, 5 times every 6 hours, and the perfusion was carried out 4 hours after the final injection. Demecolcine was given subcutaneously at a dose of 1.0 mg/kg/injection, and vinblastine was given intravenously at a dose

[8] The V × 2 carcinoma was kindly provided in 1965 by Dr. B. Clarkson of the Memorial Sloan-Kettering Cancer Center, and has been transplanted over 3½ years through 90 generations in the Surgical Research Laboratory of the Harlem Hospital Center.

[9] Mean diameter was calculated as $\frac{p+q+r}{3}$, where p, q, and r were each of the three diameters measured in three dimensions.

[10] Demecolcine: NSC-3096; colchicine, *N*-deacetyl-*N*-methyl-; Colcemid. Supplied by CIBA Pharmaceutical Co., Summit, NJ.

Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate; Velban. Supplied by Eli Lilly and Co., Indianapolis, Ind.

Nitrogen mustard: NSC-762; diethylamine, 2,2′-dichloro-N-methyl-, hydrochloride; HN_2. Supplied by Merck Sharp & Dohme, Philadelphia, Pa.

Melphalan: NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard. Supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, NY.

of 0.1 mg/kg/injection. Nitrogen mustard, at a dose of 0.3 mg/kg, was freshly dissolved in normal saline immediately before use, and was given in three divided doses at 10-minute intervals through a catheter cannulated into the femoral artery, with termination of perfusion 20 minutes after the final dose. Melphalan, at a dose of 1 mg/kg, was introduced into the oxygenator in one dose, and the perfusate was washed out of the hind limb with 30–40 ml of normal saline after the 1-hour perfusion.

According to the combination of the pretreatment and the perfusion, 92 treated rabbits were classified into eight groups (table 1). Autopsies were carried out in all cases and studies of microscopic sections were done where necessary to confirm the gross pathologic observations. "Cure" was defined as survival for more than 300 days after perfusion without any macroscopic and microscopic evidence of the carcinoma.

RESULTS

Control Perfusion

Twelve tumors perfused with blood alone grew rapidly. Their mean diameter reached 5 cm 3 weeks after perfusion (fig 1). The survival curve was steep (fig 2), and the mean survival time was short, 88.1 days (table 1). The maximum survival time was 118 days. The growth rate of the tumor, the survival curve, and the mean survival time were the same as those of the non-perfused rabbits. One case was eliminated from the data because the tumor regressed spontaneously.

Demecolcine Treatment Alone

All eight rabbits survived the serial injections with demecolcine. The tumor growth rate and the survival curve were not significantly different from those of the controls and there were no 120-day survivors (table 1, figs 1 and 2).

Vinblastine Treatment Alone

All eight animals survived treatment with vinblastine, although decreases in the white blood cell (wbc) counts and weight losses were more pronounced than in the demecolcine treatment group. In the group receiving vinblastine alone, the growth rate was slightly reduced (fig 1) and the mean survival time (128.0 days) was significantly longer than that of the controls ($P < 0.001$), but no "cures" were observed. Six of eight rabbits survived more than 120 days, but only one survived 150 days (table 1).

TABLE 1.—Ninety-two rabbits pretreated with metaphase-arresting agents before isolation perfusion with alkylating agents

Treatment				Survival rate			Mean survival time ±SD (days)
Group	Pretreatment	Perfusion	No. of animals	120 days	150 days	300 days ("cured")	
No treatment (control)	—	Blood only	12	0/12 (0%)	0/12 (0%)	0/12 (0%)	88.1±22.0
Pretreatment only	Demecolcine	—	8	0/8 (0%)	0/8 (0%)	0/8 (0%)	86.8±10.6
	Vinblastime	—	8	6/8 (75%)	1/8 (12.5%)	0/8 (0%)	128.0±17.3
Perfusion only	—	Nitrogen mustard	9	2/9 (22.2%)	0/9 (0%)	0/9 (0%)	94.0±23.9
	—	Melphalan	19	14/19 (73.7%)	12/19 (63.2%)	9/19 (47.4%)	121.8±38.1*
Pretreatment plus perfusion	Demecolcine	Nitrogen mustard	7	5/7 (71.4%)	0/7 (0%)	0/7 (0%)	116.4±20.2
	Demecolcine	Melphalan	16	13/16 (81.3%)	12/16 (75.0%)	8/16 (50.0%)	149.8±69.1*
	Vinblastine	Melphalan	13	11/13 (84.6%)	11/13 (84.6%)	8/13 (61.6%)	146.6±51.6*

*In these groups, the mean survival time and standard deviation were calculated excluding "cured" animals.

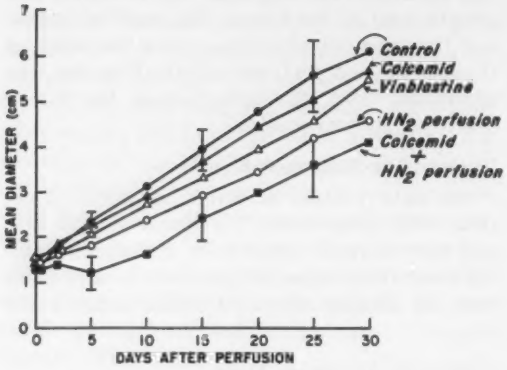

FIGURE 1.—Effect of demecolcine, vinblastine, nitrogen mustard perfusion, and demecolcine + nitrogen mustard perfusion on V × 2 carcinoma of rabbit. Standard deviations are shown in the control group and the demecolcine + nitrogen mustard perfusion group.

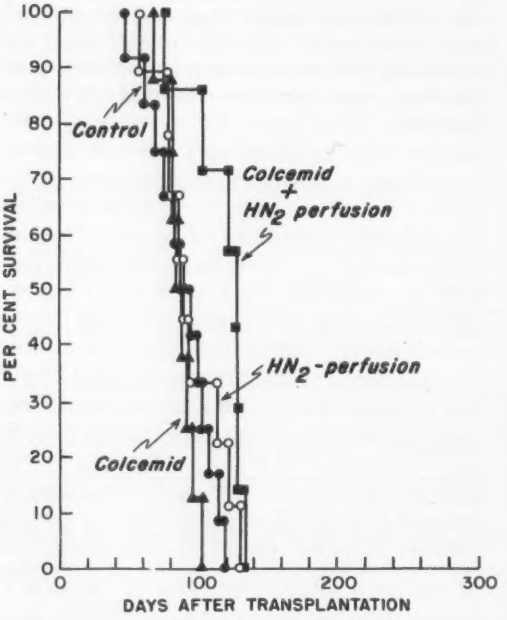

FIGURE 2.—Survival curves for controls and groups treated with demecolcine only, nitrogen mustard perfusion only, and demecolcine + nitrogen mustard perfusion.

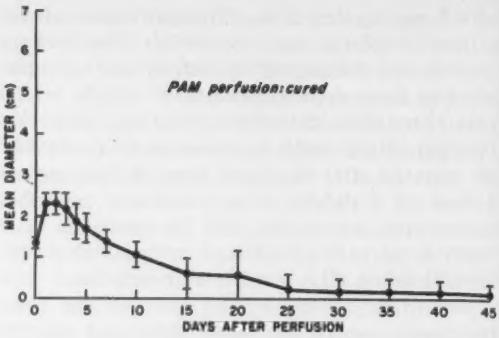

FIGURE 3.—Mean diameter and standard deviation of V × 2 carcinoma immediately before and after melphalan perfusion in 9 "cured" animals. The tumor showed edematous enlargement for the first few days after perfusion.

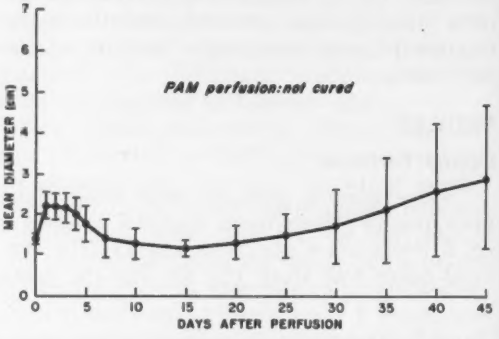

FIGURE 4.—Mean diameter and standard deviation of V × 2 carcinoma immediately before and after melphalan perfusion in 10 "non-cured" animals. The shrinkage of the tumor after the edema had subsided was not as prominent as in "cured" animals, and the tumor started to grow again 2–3 weeks after the perfusion.

Nitrogen Mustard Perfusion Alone

Isolation perfusion with 0.3 mg/kg nitrogen mustard did not cause skin necrosis or paralysis of the leg in any of the nine rabbits treated. Slight edema was observed around the tumor for the first few days after the perfusion but it subsided in 1 week. As shown in figure 1, there was a delay in tumor growth, but the mean survival time (94.0 days) did not differ significantly from that observed in the control animals. Survival for 120 days was obtained in two rabbits, though none of the animals survived 150 days (table 1, fig 2).

Melphalan Perfusion Alone

Of 24 rabbits perfused with 1 mg/kg of melphalan, two died a few days after perfusion and severe local necrosis developed in three. These five cases were omitted from the data. In the remaining 19 animals, the function of the perfused legs was well preserved, though slight to moderate atrophy was observed in some rabbits after the edema subsided. In nine of 19 cases, "cure" was obtained (table 1, fig 3). The remaining ten animals had delayed tumor growth (fig 4) and prolonged survival time (121.8 days) (table 1, figs 5 and 6).

Combination of Demecolcine Pretreatment and Nitrogen Mustard Perfusion

All seven animals in this group survived the treatment without any local damage. Though all the rabbits died of the cancer, the tumors grew slightly slower than with notrogen mustard perfusion alone (fig 1) and the mean survival time (116.4 days) was longer than with nitrogen mustard perfusion alone ($P < 0.1$). Five of seven animals survived more than 120 days, but no 150-day survival was obtained (table 1, fig 2).

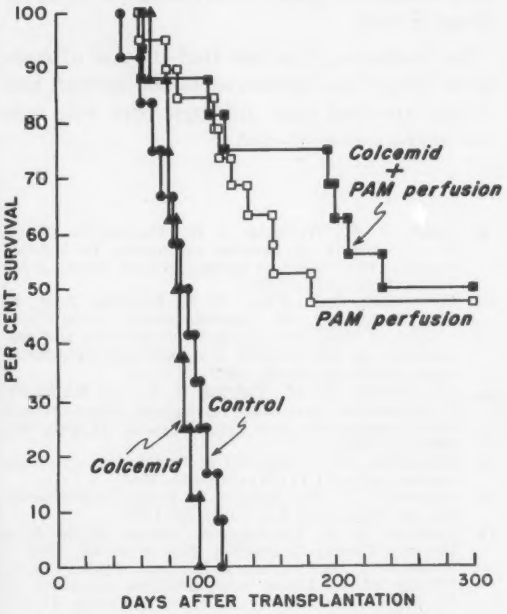

FIGURE 5.—Survival curves for controls and groups treated with demecolcine only, melphalan perfusion only ("cures" obtained), and demecolcine + melphalan perfusion ("cures" obtained).

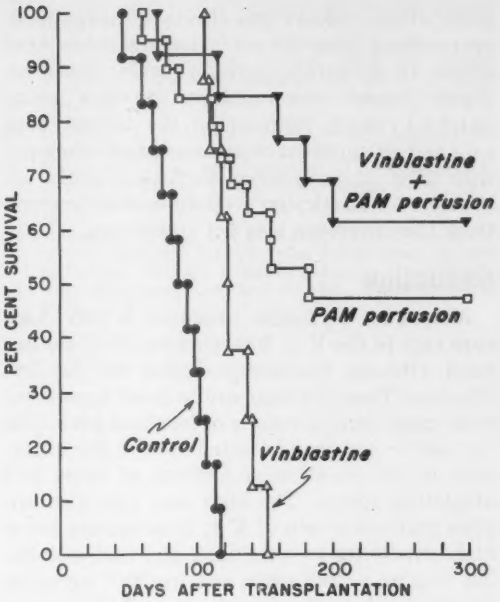

FIGURE 6.—Survival curves for controls and groups treated with vinblastine only, melphalan perfusion only ("cures" obtained), and vinblastine + melphalan perfusion ("cures" obtained). Of all 8 groups, vinblastine + melphalan perfusion yielded the best results.

Combination of Demecolcine Pretreatment and Melphalan Perfusion

Eight "cures" were obtained in 16 cases. Although the survival curve appears to be improved (fig 5), the curability of 50% was not appreciably different from that of melphalan perfusion alone, the growth curve being almost the same. A severe soft tissue necrosis developed in one rabbit in this group and the animal was eliminated from the data.

Combination of Vinblastine Pretreatment and Melphalan Perfusion

All 13 animals initially survived the treat-

ment without severe side effects, although moderate edema after the perfusion and subsequent slight to moderate atrophy were observed. Eight "cures" were obtained in this group (61.6%) which represented the largest cure rate and an apparent improvement as compared with that of melphalan perfusion alone, although statistically (Kolmogorov-Smirnov test) the difference was not significant.

DISCUSSION

Melphalan perfusion produced a very high cure rate in the V × 2 carcinoma. On the other hand, nitrogen mustard perfusion was far less effective. These findings are in good agreement with many clinical results of localized perfusion for cancer and may be attributed to the difference in the duration of activity of these two alkylating agents. The high cure rate also implies that our strain of V × 2 carcinoma has a high sensitivity to melphalan and that melphalan acts as a "cell cycle non-specific" agent at the extremely high concentration offered by the localized perfusion.

Vinblastine was superior to demecolcine as a means of pretreatment, with 75% of the animals surviving 120 days, although none was cured of the disease by 300 days. This may be explained by the fact that vinblastine induces metaphase arrest permanently, whereas the metaphase arrest induced by colchicine (NSC-757) or its derivatives is reversible (19). Vinblastine may have destroyed some population of cancer cells by the permanent metaphase arrest and, in so doing, increased the so-called growth fraction. Partial synchronization of tumor cells induced by demecolcine does not seem to have appreciably increased the sensitivity of the cells to the alkylating agents.

The work of Marmond (26) has shown the correlation of oncolytic effect and magnitude of metaphase blockade with the vinca alkaloids. In the present in vivo study using V × 2 carcinoma in rabbit hind limb, attention was directed mainly toward uncomplicated tumor growth and correlation with animal survival, rather than mitotic studies that would have required either incisional or excisional biopsy, both of which would have mitigated against the purpose of the study. Correlation of mitotic indices with DNA replication forms the essence of a subsequent paper to evaluate the effect of drugs in vivo.

In conclusion, it is felt that the use of combined drugs has influenced tumor growth and animal survival time although cure rate was not impressively affected.

REFERENCES

1. CREECH, O., JR., KREMENTZ, E. T., RYAN, R. F., ET AL. Chemotherapy of cancer: regional perfusion utilizing an extracorporeal circuit. Ann Surg 148:616–632, 1958.
2. CREECH, O., JR., KREMENTZ, E. T., RYAN, R. F., ET AL. Experiences with isolation-perfusion technics in the treatment of cancer. Ann Surg 149:627–639, 1959.
3. STEHLIN, J. S., JR., CLARK, R. L., JR., WHITE, E. C., ET AL. Regional chemotherapy for cancer: experiences with 116 perfusions. Ann Surg 151:605–619, 1960.
4. KREMETZ, E. T., CREECH, O., JR., and RYAN, R. F. Evaluation of chemotherapy of cancer by regional perfusion. Cancer 20:834–838, 1967.
5. WHITE, L. P. Antitumor activity of nontoxic preparations of HN_2. Proc Am Assoc Cancer Res 3:162, 1960.
6. AUSMAN, R. K., GEMMILL, S. J., and AUST, J. B. Test for the presence of alkylating agent in blood—its use in isolated organ perfusion. Proc Am Assoc Cancer Res 3:4, 1959.
7. KREMENTZ, E. T., and KNUDSON, L. The effect of increased oxygen tension on the tumoricidal effect of nitrogen mustard. Surgery 50:26–73, 1961.
8. LEATHER, R. P., and EIBERT, C. Hyperbaric oxygenation and mechlorethamine effectiveness. Arch Surg 87:144–147, 1963.
9. RYAN, R. F., WINBLAD, J. N., HOTTINGER, G. C., ET AL. Effects of various perfusates in isolated vascular beds using an extracorporeal circuit. Surg Forum 9:193–196, 1958.
10. HOTTINGER, G. C., RYAN, R. F., DELGADO, J. P., ET AL. Physiology of extracorporeal circulation: studies of blood flow, oxygen consumption and metabolism in the isolated and perfused extremity. Surg Forum 10:80–82, 1960.
11. SHINGLETON, W. W., PARKER, R. T., and MAHALEY, S. Abdominal perfusion for cancer chemotherapy with hypothermia and hyperthermia. Surgery 50: 260–265, 1961.
12. LONGMORE, D. B. Regional perfusion with cytotoxic agents. Lancet 1 (7126):673–675, 1960.
13. GOLOMB, F. M. The selection of drugs for perfusion. Cancer Chemother Rep 10:53–55, 1960.
14. MARTIN, D. S. Combination versus single drug therapy. Cancer Chemother Rep 10:57–59, 1960.
15. RYAN, R. F., LOCHRIDGE, H. L., and JACKSON, D. A. Effects of colchicine and nitrogen mustard on Ehrlich's ascitic tumor mice. Surg Forum 11:47–49, 1960.
16. HORTON, J., OLSON, K. B., GEHRT, P., ET AL. Combination therapy with 5-fluorouracil (NSC–19893), mitomycin C (NSC-26980), vincristine (NSC-67574), and thioTEPA (NSC-6396) for advanced cancer. Cancer Chemother Rep 49:59–61, 1965.

17. NATHANSON, L., HALL, T. C., DEDERICK, M. M., ET AL. Initial pharmacologic studies of three types of combination chemotherapy. Cancer Chemother Rep 50:259–264, 1966.
18. GOLDIN, A., VENDITTI, J. M., MANTEL, N., ET AL. Evaluation of combination chemotherapy with three drugs. Cancer Res 28:950–960, 1968.
19. ARMSTRONG, J. G. Vincristine (NSC-67574) Symposium—summary and prospectives. Cancer Chemother Rep 52:527–535, 1968.
20. ANDREWS, J. R. Commentary: Combined cancer radiotherapy and chemotherapy: the relevance of cell population kinetics and pharmacodynamics. Cancer Chemother Rep 53:313–324, 1969.
21. BASERGA, R. Biochemistry of the cell cycle: a review. Cell Tissue Kinet 1:167–191, 1968.
22. SCHABEL, F. M., JR. The use of tumor growth kinetics in planning "curative" chemotherapy of advanced solid tumors. Cancer Res 29:2384–2389, 1969.
23. MORI, S., CLARKSON, B., MASLE, E. T., ET AL. An experimental perfusion system using the rabbit Vx-2 carcinoma. I. Studies on the effect of drug perfusions and irradiation. J Surg Res 2:268–282, 1962.
24. LARIONOV, L. F. Cancer Chemotherapy, 1st ed. New York, Pergamon Press, Inc., 1965, p 35.
25. HAUSCHKA, T. Tissue genetics of neoplastic cell population. Canad Cancer Conf (Begg, R. W., ed). New York, Academic Press, Inc., 1957, vol 2, p 305.
26. MARMONT, A. M., and DAMASIO, E. E. The effects of two alkaloids derived from *Vinca rosea* on the malignant cells of Hodgkin's disease, lymphosarcoma and acute leukemia in vivo. Blood 29:1–21, 1967.

Success and Failure in the Treatment of Solid Tumors. III. "Cure" of Metastatic Lewis Lung Carcinoma With Methyl-CCNU (NSC-95441) and Surgery-Chemotherapy [1, 2, 3]

J. G. Mayo, W. R. Laster, Jr., C.M. Andrews, and F. M. Schabel, Jr. [4]

SUMMARY

Subcutaneously (sc) implanted Lewis lung carcinoma grows in a Gompertzian manner and metastasizes to the lungs of BDF_1 mice before the primary tumor reaches 0.4 g in size. At 0.35 g the primary Lewis lung tumor has a doubling time of 2.4 days, and 6.4-g tumors have a doubling time of 23 days (average values). Studies by Karrer and Humphreys have shown that primary plus metastatic Lewis lung tumor cannot be cured by surgical treatment or cyclophosphamide alone. However, when the solid primary tumor was removed surgically, systemic therapy (cyclophosphamide) resulted in long-term tumor-free survivors. A large number of drugs have been screened against early and moderately advanced Lewis lung tumor implanted sc. Cyclophosphamide and a number of nitrosoureas markedly inhibited growth of early Lewis lung tumor, but only methyl-CCNU caused complete regression and "cures" (34%) of 400-mg Lewis lung tumors. The combination of methyl-CCNU plus cyclophosphamide was more effective in "curing" Lewis lung tumor than methyl-CCNU alone. When used alone, cyclophosphamide and methyl-CCNU were equally quite effective in "curing" widely disseminated Lewis lung tumor cells in the absence of a solid primary tumor (ie, when single doses were administered 2–10 days after intravenous inoculation of 10^6 tumor cells). Thus, the superiority of methyl-CCNU over cyclophosphamide is limited to its greater effectiveness against "solid tumor" masses. After Lewis lung tumors have metastasized, surgical removal of the primary tumor significantly improves the "cure" of the remaining metastatic disease by methyl-CCNU, cyclophosphamide, or methyl-CCNU plus cyclophosphamide. Surgical treatment followed by the drug combination appears to provide greater than 50% "cures" of quite advanced Lewis lung carcinoma.

[Cancer Chemother Rep 56:183–195, 1972]

Karrer and Humphreys (1,2) have shown that primary plus metastatic Lewis lung carcinoma in BDF_1 mice is not curable by surgical treatment alone or cyclophosphamide alone. However, when the solid primary tumor was removed surgically, systemic treatment with

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[2] Supported by contracts NIH-71-2098, PH43-66-29, and NIH-71-2021 from Chemotherapy, National Cancer Institute, and a grant from the Kettering Family.
[3] Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.
[4] Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

cyclophosphamide resulted in long-term tumor-free survivors.

We have observed that methyl-CCNU is unusually effective against the refractory Lewis lung solid tumor (3). This compound was synthesized by Johnston et al (4,5) in 1966 and shown to be effective against L1210 leukemia in animals inoculated intraperitoneally (ip), intravenously (iv), or intracerebrally (ic).

This report presents therapeutic trial results obtained in the Lewis lung carcinoma system with methyl-CCNU, cyclophosphamide, and the combination of methyl-CCNU plus cyclophosphamide when such chemotherapy was used alone and after surgical removal of the primary Lewis lung tumor.

EXPERIMENTAL RESULTS

Growth Rate and Kinetic Parameters of Lewis Lung Carcinoma

As in previous papers on the treatment of model tumor systems (6–9), we present some background data on the growth rate of the tumor used and the proliferative behavior of the tumor cells. We believe that such information is helpful in assessing the potential for carry-over of therapeutic results to different tumor systems in the same and different hosts.

The average growth rate of the solid Lewis lung tumor arising from subcutaneous (sc) implantation of approximately 25-mg trocar fragments can be judged from the pooled data shown in table 1 (9).

TABLE 1.—Average growth rate of the solid Lewis lung tumor*

Days postimplant	Average primary tumor weight† (mg)	Average doubling time at weights indicated (days)
6	350	2.4
8	620	2.9
12	1530	4.4
16	2920	6.7
20	4615	11
24	6400	23

*Tumors arising from sc implantation of approximately 25-mg trocar fragments.

† Weights of all sc tumors were obtained by converting caliper measurements in millimeters to mass (milligrams) using the formula for the volume of a spheroid $\frac{a \times b^2}{2} = W$, where a = length of tumor and b = width of tumor.

Ic titrations have shown that the doubling time of relatively small numbers of Lewis lung tumor cells (10^2–10^6) is about 1 day (fig 1).

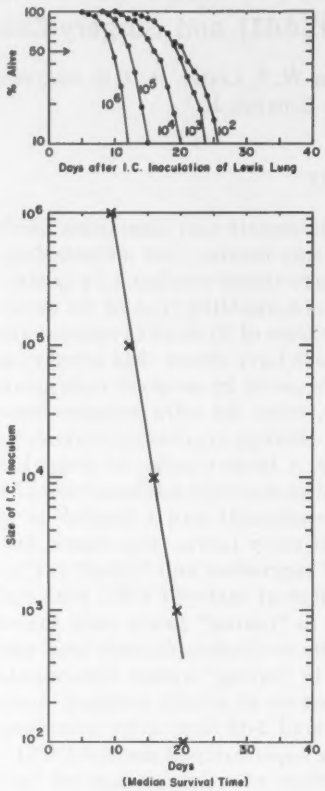


FIGURE 1.—Doubling time of relatively small numbers of Lewis lung tumor cells injected ic.

Size of inoculum	Median survival time of dying animals (days)	Surviving animals	Doubling time (days) $\left(\frac{\text{difference in days}}{3.32}\right)$*
10^6	9.5	0/98	0.81
10^5	12.5	0/99	1.1
10^4	16	0/98	1.1
10^3	19.5	30/96†	
10^2	(20.5)	71/91†	

*Difference in median survival of animals receiving inocula varying in size by 10-fold divided by the number of doublings in a decade (3.32) indicates a doubling time of about 1 day.

† These surviving fractions suggest that about 0.1%–1.0% of the tumor cells were clonogenic on ic passage.

TABLE 2.—Effects of delay in surgical treatment of sc Lewis lung tumors on the survival pattern of BDF_1 mice

Day of surgical treatment (postimplant)	Median wt tumor weight (mg)	Day of death (postimplant)																													Tumor-free survivors on Day 120	Median survival time (days)	At death: No. with tumors*	At death: No. with extensive lung metastasis	At death: Median lung weight† (mg)
		13	14	16	19	22	23	24	25	26	27	28	29	30	31	32	33	35	36	37	39	40	43	44	45	48	68					
No treatment (control)	—								1	1	2	5	(5)	4	1	1												0/20	29	20/20	20/20	848
2	<60															1	1	1	1		1	1	1	1	2	1	1	6/20	39.5	13/14	14/14	860
4	<100										1	1	1					2	1	2	1		1	1		1		8/20	35.5	11/12	12/12	878
6	352						2	2	2	1	1	(5)	3	1	1	1	1											0/20	28	12/20	20/20	1073
8	604				1			2	3	3	1	6	3			1												0/20	27.5	8/20	19/20	1125
10	1150	1	1			1	1	2	1	(7)	2	3	1															0/20	26	9/20	19/20	1103
12	1522			1			1	2	(7)	5		1	1		2													0/20	25	7/20	19/20	1160
									median																								

*Recurrence at original site or adjacent nodes (more often in adjacent lymph nodes).
†Normal lung weight is about 165 mg with a range of 125–200 mg.

The pulse thymidine index of cells in 0.5-g tumors is 15% (9), and the median cycle parameters for tumor cells in cycle are as follows: T_C, 18.8 hours; T_S, 8.5 hours; T_{G_2}, 1.8 hours; and T_{G_1}, 7.5 hours.

Rate of Metastasis of Lewis Lung Carcinoma Cells

When Humphreys and Karrer (2) implanted Lewis lung tumor cells in the calf of the hind leg of BDF_1 mice and performed radical surgical amputation of the tumor-bearing limb 8, 12, or 16 days later, they observed approximately 50%, 2%, and 0 surgical "cures" respectively.

In our experience with sc implants, surgical removal of the primary tumor at 6 days (or greater) failed to provide any "cures" or increases in host survival times over untreated controls (*see* table 2). Additional data on the rate of lung involvement after sc implantation of Lewis lung tumor fragments are presented in table 3, along with results obtained after iv inoculation with suspended tumor cells.

Survival curves of animals receiving Lewis lung tumor cells inoculated by different routes are given in figure 2.

Response of Lewis Lung Carcinoma to Chemotherapy Alone

Extensive screening results obtained with early and moderately advanced Lewis lung carcinoma implanted sc show it to be refractory to many drugs when administered ip in conventional daily dose schedules (table 4). Cyclophosphamide and various nitrosoureas were the most effective agents. Methyl-CCNU stood out as the most effective single drug against moderately advanced (0.4 g) solid Lewis lung tumors.

Comparative therapeutic trials were carried out against 0.4-g primary Lewis lung tumors using methyl-CCNU, CCNU, BCNU, cyclophosphamide (table 5), and methyl-CCNU plus cyclophosphamide (table 6). The end points used were maximum tumor regression at the nadir, increase in host survival time over untreated controls, and long-term tumor-free survivors. Typical tumor regression and recur-

TABLE 3.—Lung involvement after sc and iv implantation of Lewis lung tumor cells

Days after implantation	Sc implantation of tumor fragments			Iv inoculation with 10^6 tumor cells	
	Average primary tumor weight	Average lung weights (mg)	Gross appearance of lungs	Average lung weights (mg)	Gross appearance of lungs
0 (controls)		149	Normal	183	Normal
3	<60	151	Normal	174	Normal
6	350	*		194	? Colonies
9	800	184	? Colonies	242	Colonies
12	1530	158*		241	Colonies
15	2500	163*	Colonies†	271	Colonies
18	3600	166	Colonies	658	Many colonies
21	5000	268	Many colonies	713	Many colonies
24	6400	318	Many colonies	877	Massive involvement
27	8000	292	Many colonies	All dead	
32	9200	659	Massive tumor involvement		

*Lungs were proved to be positive by assay (ie, lung suspension produced tumors on passage).
†Macroscopic colonies of tumor cells in the lung.

rence curves are presented in figures 3 and 4. Typical survival curves are given in figure 5.

The experiments summarized in table 7 were carried out to compare the effectiveness of maximum tolerated single doses (and fractions thereof) of methyl-CCNU, cyclophosphamide, CCNU, and BCNU against disseminated Lewis lung carcinoma cells in the absence of a solid primary tumor. The single doses of each drug were administered ip on Days 2, 6, 10, and 14 after iv inoculation with 10^6 cells to obtain comparisons at varying degrees of advancement of systemic disease.

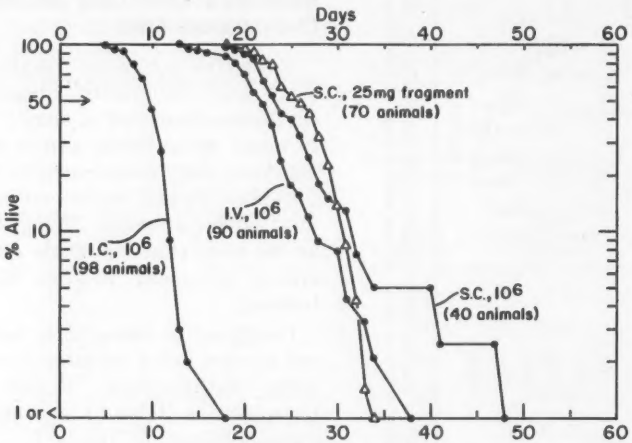


FIGURE 2.—Survival curves of BDF_1 mice receiving 10^6 suspended Lewis lung tumor cells injected by different routes vs sc implantation of small (25 mg) tumor fragments.

TABLE 4.—Primary screening results against early and moderately advanced Lewis lung carcinoma (implanted sc)*

Agent	Therapy begun on Day 1 or 2: T/C on Day 12†	Therapy begun on Day 7: inhibition or regression‡	
	Chronic	Single dose	Chronic
Methotrexate	0.65	−	−
6-MP	0.52	−	−
6-Thioguanine	0.36		−
6-Methyl MPR			+
6-Chloropurine			−
5-FU	0.51		−
5-FUDR	0.20		+
PalmO-ara-C	0.36		−
Arabinosylcytosine	0.87		
Hydroxyurea			−
Vincristine	0.63		
Vinblastine	0.99		
Prednisone			−
Daunomycin	0.56		
Actinomycin D	0.55	−	−
Bleomycin	0.06		+
Nitrogen mustard	0.65		
Alanine mustard	0.76		
D,L-Sarcolysin	0.32	−	−
Uracil mustard	0.38		
Cyclophosphamide	0	++	−
BCNU	0	+	−
CCNU		++	+
Methyl-CCNU (trans)	0	+++	+++
Methyl-CCNU (cis)	0.39	++	
Other nitrosoureas:			
NSC-88104	0.18	++	−
NSC-93184	0.02	++	++
NSC-93372		++	++
NSC-95466		++	
NSC-103548	0	++	++
NSC-102724		++	
NSC-105763	0	++	+
NSC-106767		++	++
NSC-114459	0	++	++
NSC-117624		++	
NSC-125649		++	
NSC-128303		++	
NSC-129967		++	
NSC-129668		++	

* All screening was at or near maximum tolerated doses. Many other agents of various classes were screened and provided poor response.

† T/C = treated/control tumor weight; 0 = complete inhibition of growth to Day 12.

‡ − = No evidence of tumor regression.

+ = Temporary inhibition of tumor but no regression below original tumor size.

++ = Moderate temporary regression followed by regrowth; no "cures."

+++ = Complete temporary regression; some "cures."

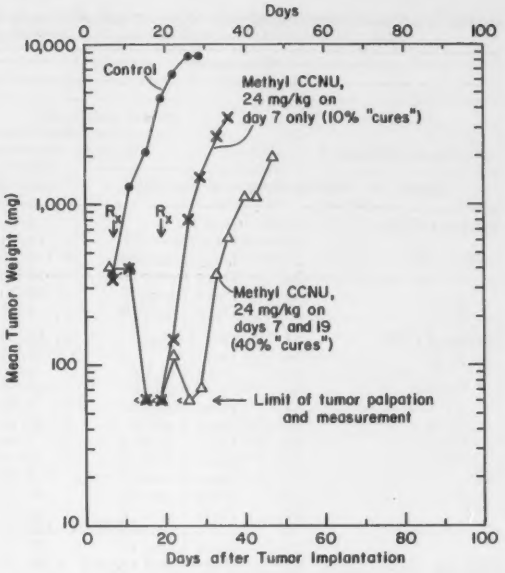


FIGURE 3.—Typical regression and recurrence of Lewis lung tumors after treatment with methyl-CCNU.

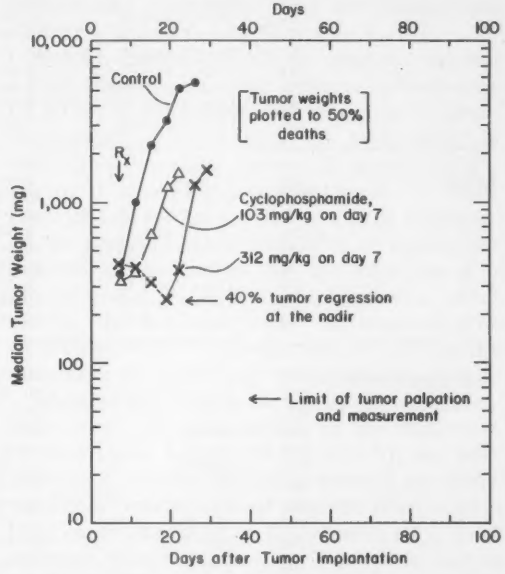


FIGURE 4.—Typical inhibition or moderate regression of Lewis lung tumors after treatment with cyclophosphamide. The moderate (40%) regression in tumor mass obtained with 312 mg/kg of cyclophosphamide implies a much greater than 40% tumor cell kill (6).

TABLE 5.—Quantitative therapeutic trials against 400-mg Lewis lung tumors: Effectiveness of methyl-CCNU, CCNU, BCNU, and cyclophosphamide with respect to regression of the primary tumor, host survival time, and "cure"

Treatment				Response				
				Maximum tumor mass regression at nadir		Median percent increase in host lifespan (including survivors)	Tumor-free survivors	
Agent	Mg/kg/dose	Schedule	Survivors in concurrent toxicity controls	Day	Median regression		Period of observation (days)	No./total
Methyl-CCNU	36	Day 7	9/10	15	Complete*	57	97	0/10
	24	Day 7	10/10	15	Complete	40		1/10
	24	Days 7 and 13	10/10	19	Complete	123		3/10
	24	Days 7 and 15	10/10	15	Complete	43		1/10
	24	Days 7 and 17	10/10	19	Complete	50		0/10
	24	Days 7 and 19	9/10	15	Complete	73		4/10
Methyl-CCNU	24	Day 7	10/10	16	69%	61	90	1/10
	16	Day 7	10/10	16	60%	13		0/10
	24	Days 7 and 8	8/10	20	Complete	70		3/10
	16	Days 7 and 8	10/10	20	Complete	18		3/10
	24	Days 7 and 9	7/10	20	Complete	>290		6/10
	16	Days 7 and 9	10/10	20	Complete	95		0/10
	24	Days 7 and 10	7/10	20	Complete	>290		5/10
	16	Days 7 and 10	10/10	20	Complete	91		0/10
	24	Days 7 and 11	9/10	20	Complete	200		4/10
	16	Days 7 and 11	10/10	20	Complete	143		2/10
	24	Days 7 and 12	10/10	20	Complete	>290		7/10
	16	Days 7 and 12	10/10	20	Complete	57		1/10
	24	Days 7 and 13	10/10	20	Complete	>290		5/10
	16	Days 7 and 13	10/10	20	66%	57		0/10
CCNU	50	Day 7	10/10	15	Complete	7	97	0/10
	33	Day 7	10/10	—	0	7		0/10
	33	Days 7 and 13	10/10	19	45%	20		0/10
	50	Days 7 and 15	10/10	15	Complete	63		0/10
	33	Days 7 and 15	10/10	—	0	0		0/10
	50	Days 7 and 17	9/10	15	72%	51		0/10
	33	Days 7 and 17	9/10	—	0	0		0/10
	50	Days 7 and 19	9/10	15	Complete	59		0/10
	33	Days 7 and 19	8/10	—	0	0		0/10
BCNU	39	Day 7	9/10	15	54%	0	97	0/10
	26	Day 7	10/10	—	0	0		0/10
	39	Days 7 and 13	10/10	15	58%	0		0/10
	26	Days 7 and 13	9/10	—	0	0		0/10
	39	Days 7 and 15	10/10	—	0	0		0/10
	26	Days 7 and 15	10/10	—	0	0		0/10
	39	Days 7 and 17	9/10	—	0	0		0/10
	26	Days 7 and 17	10/10	—	0	0		0/10
	26	Days 7 and 19	9/10	—	0	0		0/10
Cyclophosphamide	312	Day 7	10/10	19	42%	43	47	0/10
	300	Day 7	10/10	14	69%	76	63	0/10
	300	Day 7	10/10	12	68%	65	82	0/10
	230	Day 8	10/10	15	12%	52	47	0/10
	225	Day 7	10/10	14	54%	42	63	0/10
	225	Day 7	10/10	14	9%	42	58	0/10
	209	Day 7	10/10	15	14%	70	47	0/10
	150	Day 8	10/10	—	0	31	47	0/10
	150	Day 7	10/10	10	22%	10	63	0/10
	150	Day 7	10/10	—	0	42	58	0/10
	103	Day 7	10/10	—	0	0	47	0/10
	225	Days 7 and 14	10/10	22	78%	98	82	1/10
	225	Days 7, 14, 21, 28	8/10	26	83%	117	82	0/10
	150	Days 7, 14, 21, 28	10/10	12	46%	137	82	0/10

*Complete regression of 400-mg sc tumors to unpalpable dimensions; there was subsequent recurrence and progressive tumor growth in essentially all animals *except* in the long-term tumor-free survivors.

TABLE 6.—Effectiveness of the combination of methyl-CCNU plus cyclophosphamide against 400-mg Lewis lung tumors (regression of the primary tumor, host survival time, and "cures")

Treatment				Response				
				Maximum tumor mass regression at nadir		Median percent increase in host survival time (including survivors)	Tumor-free survivors	
Agent(s)	Mg/kg/dose	Schedule	Survivors in concurrent toxicity controls	Day	Median regression		Period of observation to date (days)	No./total
Methyl-CCNU	36	Day 7	10/10	18	50%	27	75	1/20
	24	Day 7	10/10	18	75%	57		2/20
Cyclophosphamide	225	Day 7	10/10	18	32%	50		0/10
	150	Day 7	10/10	—	—	25		0/10
Cyclophosphamide+ methyl-CCNU	150+24	Day 7	10/10	31	Complete	>168		16/20
	150+16	Day 7	9/10	38	Complete	>168		11/20
	100+24	Day 7	9/10	18	77%	46		6/20
	100+16	Day 7	10/10	18	64%	64		1/20
	67+24	Day 7	10/10	18	18%	18		0/20

Response of Lewis Lung Carcinoma to Surgery-Chemotherapy

Internally controlled therapeutic trials were then carried out comparing the effectiveness of surgical treatment and methyl-CCNU used alone versus surgical treatment followed by methyl-CCNU (table 8).

Similarly, the effectiveness of surgical treatment, the combination of methyl-CCNU plus cyclophosphamide, and surgical treatment followed by the combination of drugs were studied in the experiment summarized in table 9.

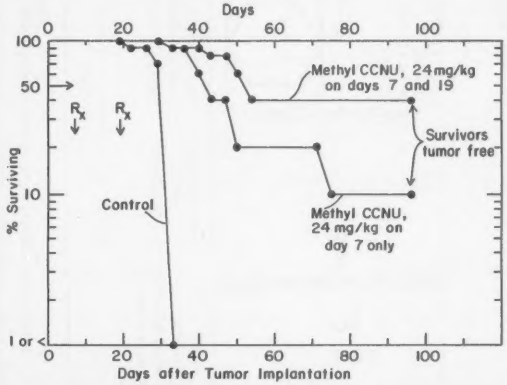


FIGURE 5.—Typical survival curves of mice bearing Lewis lung carcinoma (controls vs groups treated with methyl-CCNU).

DISCUSSION

We have been pleasantly surprised at the clear superiority of methyl-CCNU over CCNU or BCNU in the treatment of solid Lewis lung tumors and metastatic lung carcinoma cells (tables 4, 5, and 7) (fig 6).

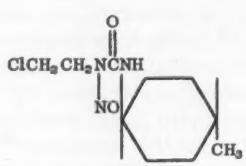
Briefly, methyl-CCNU is effective against L1210 leukemia but not quite as effective as CCNU or BCNU. From the standpoint of structure-activity relationships we have observed that the *cis*-methyl isomer of methyl-CCNU (fig 6) is somewhat less effective in the treatment of Lewis lung tumors or L1210 leukemia. At the present time we can offer no convincing theoretical explanation for the influence of a methyl group in the *trans* configuration which results in the superiority of *trans*-methyl-CCNU over CCNU and *cis*-methyl-CCNU in the treatment of Lewis lung carcinoma.

Methyl-CCNU has consistently shown superiority over cyclophosphamide in the treatment of solid Lewis lung tumors (table 5), but both methyl-CCNU and cyclophosphamide are about equally effective against disseminated Lewis lung carcinoma cells in the absence of a solid primary tumor (table 7). This phenomenon might be associated with (*a*) differences in the physical or chemical behavior of the two drugs against the same tumor cells in two different "architectural states," or (*b*) differences in the cytotoxic activity of the two agents against the

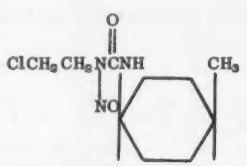
TABLE 7.—"Cure" of disseminated Lewis lung carcinoma cells in the absence of solid primary tumor (treatment started at varying periods after iv inoculation with 10^6 tumor cells)*

Agent	Single dose (mg/kg)	In terms of LD10	60-Day survivors after a single dose on the day indicated: Day 2	Day 6	Day 10	Day 14
Methyl-CCNU	36	1.0	52/66	61/86	34/84	12/88
	24	0.67	34/48	31/68	4/68	3/67
	12	0.33	15/58	2/58	0/58	4/58
Cyclophosphamide	312	1.0	15/20	8/20	9/20	0/20
	208	0.67	15/20	16/19	1/18	0/20
	103	0.33	8/10	2/10	0/10	0/20
CCNU	57	1.0	7/10	6/10	1/10	1/10
	38	0.67	2/10	0/10	0/10	0/10
	19	0.33	1/10	0/10	0/10	0/10
BCNU	41	1.0	5/10	1/10	0/10	0/10
	27	0.67	3/10	1/10	0/10	0/10
	14	0.33	0/10	1/10	0/10	0/10
			% "Cures" Day 2	Day 6	Day 10	Day 14
Methyl-CCNU	36	1.0	79	71	40	14
	24	0.67	79	46	6	4
	12	0.33	26	3	0	7
Cyclophosphamide	312	1.0	75	40	45	0
	208	0.67	75	84	6	0
	103	0.33	80	20	0	0
CCNU	57	1.0	70	60	10	10
	38	0.67	20	0	0	0
	19	0.33	10	0	0	0
BCNU	41	1.0	50	10	0	0
	27	0.67	30	10	0	0
	14	0.33	0	10	0	0

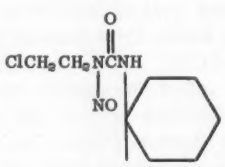
*Median lifespan of animals receiving 10^6 Lewis lung tumor cells iv is usually about 21 days. There were no survivors among concurrent control animals receiving 10^6 tumor cells.



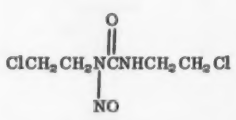
Methyl-CCNU
(trans form)



cis-Methyl-CCNU



CCNU



BCNU

FIGURE 6.—Chemical structures of methyl-CCNU (*trans* form; used in the experiments reported in this paper), *cis*-methyl-CCNU, CCNU, and BCNU.

TABLE 8.—Internal comparisons of the effects of surgical treatment, methyl-CCNU, and surgical treatment followed by methyl-CCNU on the survival time and "cure" of animals bearing metastatic Lewis lung carcinoma

Approximate primary tumor mass at start of therapy (mg)	Treatment		Response				
					Tumor-free survivors		
	Surgery (days postimplant)	Methyl-CCNU 24 mg/kg/dose on days indicated	Median host lifespan (days)	% ILS	Period of observation (days)	No./total	Comment
Control	—	—	32	—	188	0/10	Surgery-chemotherapy was more effective than methyl-CCNU alone
400	7	—	29	0	188	0/10	
	—	Day 8	48	50	188	0/10	
	—	Days 8 and 15	90	184	188	3/10	
	7	Day 8	65	104	188	4/10	
	7	Days 8 and 15	116	266	188	4/10	
Control	—	—	33	—	188	0/10	Surgery-chemotherapy was more effective than methyl-CCNU alone
1000	12	—	31	0	188	0/10	
	—	Day 13	49	48	188	0/10	
	—	Days 13 and 20	61	84	188	0/10	
	12	Day 13	57	72	188	0/9	
	12	Days 13 and 20	67	103	188	3/10	
Control	—	—	25	—	188	0/10	Metastatic disease was too far advanced when surgery-chemotherapy was started
2000	16	—	30	18	188	0/10	
	—	Days 17 and 24	48	92	188	0/10	
	16	Day 17	41	64	188	0/10	
	16	Days 17 and 24	59	136	188	0/10	
Control	—	—	32	—	188	0/10	Metastatic disease was too far advanced when surgery-chemotherapy was started
3000	21	—	32	0	188	0/10	
	—	Day 22	27	0	188	0/10	
	—	Days 22 and 29	34	7	188	0/10	
	21	Day 22	36	14	188	0/10	
	21	Days 22 and 29	48	52	188	0/10	
Control	—	—	31	—	125	0/10	Surgery-chemotherapy was more effective than methyl-CCNU alone
400	7	—	28	0	125	0/10	
	—	Days 8 and 15	62	103	125	0/10	
	7	Days 8 and 15	69	126	125	4/10	
Control	—	—	31	—	125	0/10	Surgery-chemotherapy was slightly more effective than methyl-CCNU alone
1000	12	—	28	0	125	0/10	
	—	Days 13 and 20	50	63	125	0/10	
	12	Days 13 and 20	55	78	125	1/10	
Control	—	—	30	—	125	0/8	Surgery-chemotherapy was more effective than methyl-CCNU alone
1000	12	—	27	0	125	0/8	
	—	Day 12	45	50	125	0/8	
	—	Days 12 and 19	51	72	125	0/8	
	12	Day 12	104	252	125	3/8	
	12	Days 12 and 19	27	0	125	2/8	

TABLE 8.—Internal comparisons of the effects of surgical treatment, methyl-CCNU, and surgical treatment followed by methyl-CCNU on the survival time and "cure" of animals bearing metastatic Lewis lung carcinoma—Continued

Approximate primary tumor mass at start of therapy (mg)	Treatment		Response				
					Tumor-free survivors		
	Surgery (days postimplant)	Methyl-CCNU 24 mg/kg/dose on days indicated	Median host lifespan (days)	% ILS	Period of observation (days)	No./total	Comment
Control	—	—	31	—	125	0/8	Surgery-chemotherapy was more effective than methyl-CCNU alone for the group that received a single dose
2000	16	—	28	0	125	0/8	
	—	Day 16	45	47	125	0/8	
	—	Days 16 and 23	65	111	125	0/8	
	16	Day 16	57	85	125	2/8	
	16	Days 16 and 23	24	0	125	0/8	
Control	—	—	29	—	175	0/20	Surgery-chemotherapy was more effective than methyl-CCNU alone
1000	12	—	28	0	175	0/20	
	—	Days 13 and 20	59	103	175	0/20	
	12	Days 13 and 20	70	141	175	8/20	
Control	—	—	29	—	175	0/20	Surgery-chemotherapy was more effective than methyl-CCNU alone
1000	12	—	27	0	175	0/20	
	—	Days 13 and 20	58	100	175	0/20	
	12	Days 13 and 20	76	161	175	8/20	
Control	—	—	29	—	175	0/20	Surgery-chemotherapy was more effective than methyl-CCNU alone
1000	12	—	29	—	175	0/20	
	—	Days 13 and 20	62	112	175	0/20	
	12	Days 13 and 20	45	53	175	7/20	

TABLE 9.—Effects of surgical treatment followed by methyl-CCNU plus cyclophosphamide on advanced lung carcinoma

Average primary tumor mass at start of therapy (mg)	Surgery (days post-implant)	Chemotherapy: Agent	Mg/kg/dose	Schedule	Survivors in concurrent toxicity controls	Response: Median host lifespan: Days	% ILS	Tumor-free survivors: Period of observation (days)	No./total
Controls	—	—	—	—	—	24	—	80*	0/10
1000	12	—	—	—	—	28	17		0/10
1000	—	Methyl-CCNU	24	Day 13	10/10	29	21		0/10
	—	Methyl-CCNU	24	Days 13 and 20	10/10	46	92		1/10
	—	Cyclophosphamide	150	Day 13	10/10	18	0		0/10
	—	Cyclophosphamide	150	Days 13 and 20	10/10	25	4		0/10
	—	Methyl-CCNU+cyclophosphamide	24+150	Day 13	9/10	>53	>121		0/10
	—	Methyl-CCNU+cyclophosphamide	24+100	Day 13	10/10	>53	>121		5/10
1000	12	Methyl-CCNU	24	Day 13	*See* above	40	67	80	0/10
	12	Methyl-CCNU	24	Days 13 and 20		49	104		0/10
	12	Cyclophosphamide	150	Day 13		45	88		0/10
	12	Cyclophosphamide	150	Days 13 and 20		>53	>121		7/10
	12	Methyl-CCNU+cyclophosphamide	24+150	Day 13		>53	>121		7/10
	12	Methyl-CCNU+cyclophosphamide	24+100	Day 13		>53	>121		7/10
Controls	—	—	—	—	—	30	—	80*	0/10
2000	15	—	—	—	—	30	0		0/10
2000	—	Methyl-CCNU	24	Day 16	8/8	18	0		2/10
	—	Methyl-CCNU	24	Days 16 and 23	8/8	30	0		2/10
	—	Cyclophosphamide	150	Day 16	8/8	18	0		0/10
	—	Cyclophosphamide	150	Days 16 and 23	8/8	19	0		0/10
	—	Methyl-CCNU+cyclophosphamide	24+150	Day 16	6/8	20	0		0/8
	—	Methyl-CCNU+cyclophosphamide	24+100	Day 16	8/8	19	0		0/10
2000	15	Methyl-CCNU	24	Day 16	*See* above	36	20	80	0/10
	15	Methyl-CCNU	24	Days 16 and 23		>53	>77		5/10
	15	Cyclophosphamide	150	Day 16		37	23		0/10
	15	Cyclophosphamide	150	Days 16 and 23		48	60		3/10
	15	Methyl-CCNU+cyclophosphamide	24+150	Day 16		>53	>77		7/10
	15	Methyl-CCNU+cyclophosphamide	24+100	Day 16		>53	>77		6/10

*Experiment still in progress.

same tumor cells in somewhat different kinetic states.

The response of moderately advanced Lewis lung tumors to the combination of methyl-CCNU plus cyclophosphamide was superior to that observed with either drug alone (table 6; see also table 5). There is considerable precedent for the potential usefulness of this combination. BCNU plus cyclophosphamide has been shown to be potentiating against advanced transplanted AK lymphoma (10) and L1210 leukemia (11). BCNU-resistant L1210 leukemia cells show no cross resistance to cyclophosphamide.[5] Before the present studies, it had been observed that methyl-CCNU plus cyclophosphamide was potentiating in therapeutic trials against the refractory B16 melanoma in mice (D. P. Griswold, Jr., personal communication).

In extension of the studies of Humphreys and Karrer (2), we have observed that surgical removal of the solid primary Lewis lung tumor significantly improves the chances of "cure" of the remaining disseminated disease by methyl-CCNU (table 8) or methyl-CCNU plus cyclophosphamide (table 9). A summary of the end results obtained in the present studies is given in table 10.

Finally, the combination of methyl-CCNU plus cyclophosphamide seems worthy of consideration for trial against inoperable human neoplasms. Even in metastatic disease generally considered inoperable, surgical removal of the major solid tumor mass(es) might be warranted if followed by effective systemic chemotherapy.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Alanine mustard: NSC-17663; alanine, *N,N*-bis(2-chloroethyl)-, hydrochloride, DL-
Arabinosylcytosine: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C; cytosine arabinoside
BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

[5] Schabel, F. M., Jr. BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea]. Development, antitumor activity, and mechanism of action. *In* An Appraisal of 5-Fluorouracil and BCNU (Carter, S. K., ed). Proc Chemother Conf, Chemother Solid Tumors. Prepared by the Cancer Therapy Evaluation Branch, Chemotherapy, National Cancer Institute, Bethesda, Md, 1970, pp 159–180.

TABLE 10.—Summary to date: response of Lewis lung carcinoma at varying stages of advancement to surgical treatment alone, chemotherapy alone, and surgical treatment plus chemotherapy

Days after sc implantation	Primary tumor weight (mg)	Metastatic	Long-term tumor-free survivors							
			Surgical treatment alone	Chemotherapy alone			Surgical treatment + chemotherapy			
				Methyl-CCNU (2 doses)	Cyclophosphamide (1 or more doses)	Methyl-CCNU + cyclophosphamide (1 dose)	Surgical treatment + methyl-CCNU	Surgical treatment + cyclophosphamide	Surgical treatment + methyl-CCNU + cyclophosphamide
7	400	Yes	0/40	41/120	1/120	27/40	8/20	—/20	—/20*
12	1000	Yes	0/108	1/98	0/20	5/20	29/98	7/20	14/20*
16	2000	Yes	0/38	2/34	0/20	0/18	5/28	3/20	13/20*
21	3000	Yes	0/10	0/10	—/—	—/—	0/10	—/—	—/—
			Percentages						
			Surgical treatment	Chemotherapy			Surgical treatment + chemotherapy		
7	400	Yes	0	34	1	68	(40)*	—	—
12	1000	Yes	0	1	0	24	30	35	70*
16	2000	Yes	0	6	0	0	18	15	65*
21	3000	Yes	0	0	—/—	—/—	0	—/—	—/—

*Experiment in progress; observation for 80 days.

CCNU: NSC-79037; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
6-Chloropurine: NSC-744
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Daunomycin: NSC-82151
5-Fluorouracil: NSC-19893; 5-FU
5-FUDR: NSC-27640; uridine, 5-fluoro-2'-deoxy-
Hydroxyurea: NSC-32065
6-Mercaptopurine: NSC-755; purine-6-thiol, monohydrate; 6-MP
Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; Amethopterin
Methyl-CCNU: NSC-95441; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*-
6-Methylmercaptopurine riboside: NSC-40774; 9*H*-purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate; 6-methyl MPR
Nitrogen mustard: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride
PalmO-ara-C: NSC-135962; cytosine, 1-β-D-arabinofuranosyl-, 5'-palmitate
Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Sarcolysin: NSC-14210; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, DL-
6-Thioguanine: NSC-752; purine-6-thiol, 2-amino-
Uracil mustard: NSC-34462; uracil, 5-[bis(2-chloroethyl)-amino]-
Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; leurocristine, sulfate (1:1)
NSC-88104: urea, 3-2-chlorocyclohexyl)-1-(2-chloroethyl)-1-nitroso-, *trans*-
NSC-93184: urea, 1-(2-chloroethyl)-3-(2-fluoroethyl)-1-nitroso-, mixt. with urea, 3-(2-chloroethyl)-1-(2-fluoroethyl)-1-nitroso-
NSC-93372: urea, 1,1'-(*trans*-1,4-cyclohexylene)bis[3-(2-chloroethyl)-3-nitroso-
NSC-95466: urea, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso-
NSC-102724: cyclohexanecarboxylic acid, *cis*-3-[3-(2-chloroethyl)-3-nitrosoureido]-, ethyl ester
NSC-103548: cyclohexanecarboxylic acid, *trans*-4-[3-(2-chloroethyl)-3-nitrosoureido]-, ethyl ester
NSC-105763: urea, 1-(2-chloroethyl)-1-nitroso-3-(tetrahydrothiopyran-4-yl)-, *S,S*-dioxide
NSC-106767: urea, 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydrothiopyran-4-yl)-, *S,S*-dioxide
NSC-114459: urea, 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydro-2*H*-thiopyran-4-yl)-
NSC-117624: urea, 1-(2-chloroethyl)-3-(4-hydroxycyclohexyl)-1-nitroso-, acetate (ester)
NSC-125649: urea, 1-(2-fluoroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
NSC-128303: urea, 1-(2-chloroethyl)-3-*m*-dithian-5-yl-1-nitroso-
NSC-129967: urea, 1-(2-chloroethyl)-3-(4-ethylcyclohexyl)-1-nitroso-, mixt. with 3-(2-chloroethyl)-1-(4-ethylcyclohexyl)-1-nitrosourea
NSC-129968: urea, 3-(2-adamantyl)-1-(2-chloroethyl)-1-nitroso-

REFERENCES

1. KARRER, K., and HUMPHREYS, S. R. Continuous and limited courses of cyclophosphamide (NSC-26271) in mice with pulmonary metastasis after surgery. Cancer Chemother Rep 51:439–449, 1967.
2. HUMPHREYS, S. R., and KARRER, K. Relationship of dose schedules to the effectiveness of adjuvant chemotherapy. Cancer Chemother Rep 54:379–392, 1970.
3. LASTER, W. R., JR., MAYO, J. G., ANDREWS, C. M., ET AL. Lewis lung carcinoma as an experimental model for studies with anticancer drugs as surgical adjuvants. Proc Am Assoc Cancer Res 12:7, 1971.
4. JOHNSTON, T. P., MCCALEB, G. S., OPLIGER, P. S., ET AL. The synthesis of potential anticancer agents. XXXVI. *N*-nitrosoureas. II. Haloalkyl derivatives. J Med Chem 9:892–911, 1966.
5. JOHNSTON, T. P., MCCALEB, G. S., OPLIGER, P. S., ET AL. Synthesis of potential anticancer agents. 38. *N*-nitrosoureas. 4. Further synthesis and evaluation of haloethyl derivatives. J Med Chem 14:600–614, 1971.
6. GRISWOLD, D. P., JR., SCHABEL, F. M., JR., WILCOX, W. S., ET AL. Success and failure in the treatment of solid tumors. I. Effects of cyclophosphamide (NSC-26271) on primary and metastatic plasmacytoma in the hamster. Cancer Chemother Rep 52:345–387, 1968.
7. LASTER, W. R., JR., MAYO, J. G., SIMPSON-HERREN, L., ET AL. Success and failure in the treatment of solid tumors. II. Kinetic parameters and "cell cure" of moderately advanced carcinoma 755. Cancer Chemother Rep 53:169–188, 1969.
8. SKIPPER, H. E., SCHABEL, F. M., JR., and WILCOX, W. S. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 35:1–111, 1964.
9. SIMPSON-HERREN, L., and LLOYD, H. H. Kinetic parameters and growth curves for experimental tumor systems. Cancer Chemother Rep 54:143–174, 1970.
10. VALERIOTE, F. A., BRUCE, W. R., and MEEKER, B. E. Synergistic action of cyclophosphamide and 1,3-bis(2-chloroethyl)-1-nitrosourea on a transplanted murine lymphoma. J Natl Cancer Inst 40:935–950, 1968.
11. SCHABEL, F. M., JR. Improvement of therapeutic activity of antileukemic agents in experimental systems by varying dose schedules and by combination therapy. *In* Cancer Chemotherapy. Dose Schedules and Combinations (Hall, T. C., and Karrer, K., eds). Vienna, Verlag der Wiener Medizinischen Akademie, 1968, pp 65–82.

Bleomycin (NSC-125066): Phase I Clinical Study [1, 2]

Roland W. Sonntag [3]

SUMMARY

A phase I evaluation of bleomycin was carried out in 22 patients. Two dosage schedules were used: 30 mg (average, 0.45 mg/kg) given intravenously twice weekly as suggested in Japanese studies, and 15 mg (average, 0.25 mg/kg) given daily. Treatment was continued on both regimens until definite toxicity was noted. Toxic effects were seen in 19 patients. The most common forms of toxicity were skin changes and alopecia, fever or chills, and stomatitis; nausea and vomiting or hematologic changes were less commonly observed. Objective tumor regression was noted in six of the 19 patients treated until toxicity occurred.

[Cancer Chemother Rep 56:197–205, 1972]

Bleomycin is an antibiotic which was isolated from a strain of *Streptomyces verticillus* by H. Umezawa et al in 1966 (1). It was clearly differentiated from the previously known agent, phleomycin (NSC-61586), and was less toxic in animal tests (1–4). The preparation can be further separated into bleomycin A with seven components (A_1–A_6 and A'_2) and bleomycin B with six components (B_1–B_6) (5). These individual constituents have various toxicities and are differently metabolized. The substance clinically used contains at least 50% (55%–65%) bleomycin A_2, 20%–30% B_2, less than 3% B_4, 1%–10% A'_2, and 1%–10% A_1. The A_2 fraction, which comprises the greatest portion of the clinically used product, was further analyzed by hydrolysis and was found to be composed of seven components (6,7). Of these, four amino acids are new compounds and were isolated for the first time. Further chemical studies revealed sugar moieties (8).

The bleomycins have been shown to have a wide spectrum of antibacterial action in concentrations of 0.4–6.2 μg/ml as well as antifungal activity in concentrations of 1.5–12.5 μg/ml (4,9). Antitumor activity was shown with Ehrlich carcinoma (solid and ascitic) as well as with mouse Sarcoma 180 (4). In the mouse, doses of 62.5 mg/kg were well tolerated when given intravenously (iv) and intraperitoneally. In rabbits, 150 mg/kg given iv produced slight weight decreases but no deaths. Bleomycin was rapidly reabsorbed after subcutaneous administration and subsequently rapidly excreted in the urine. After 72 hours, the urine contained less than 1 μg/ml (4).

The highest tissue concentrations were found in the lungs, skin, and Ehrlich carcinoma tissue. High renal concentrations were not considered valid in view of the high urinary excretion rate (4,10). Tissue distribution studies, using both tritiated copper containing bleomycin and the cylinder plate method with *Bacillus subtilis*, showed that bleomycin was inactivated in all tissues except the skin, lungs, urinary tract, peritoneum around the injection site, and carcinoma tissue (10).[4] Antibacterial activity was

[1] Received May 3, 1971; revised Oct 6, 1971.

[2] Bleomycin was supplied by Mr. P. W. Klaaborg, Lundbeck, Inc., Zürich-Dübendorfstrasse, Zürich, Switzerland, representative of Lundbeck Pharmaceuticals, Copenhagen, Denmark.

[3] Oncology Station, Inselspital, University of Bern, 3008 Bern, Switzerland.

[4] Umezawa, H., Takeuchi, T., Hori, S., et al. Studies of mechanism of antitumor effect of bleomycin. Unpublished results.

not noted in sarcoma tissue. In contrast to carcinoma growth, sarcoma growth was not inhibited by bleomycin.[4] The therapeutic effect of bleomycin, therefore, seems to be correlated with its concentration, and inversely with its inactivation in tumor tissue. Differences in the distribution and inactivation of the various bleomycin fractions (A_2, B_2, and A_5) have also been described (10). In vitro inactivation of bleomycin has been seen with sulfhydryl compounds, hydrogen peroxide, and ascorbic acid. This inactivation process was potentiated by the addition of various nucleotides.

Bleomycin was first thought to act by inhibition of DNA synthesis (11). Further studies with synchronized HeLa cell cultures, however, showed greater inhibition of cell division than could be accounted for by the relatively minimally reduced thymidine incorporation (12). It now would seem that bleomycin acts by direct combination with DNA in the presence of a sulfhydryl compound or hydrogen peroxide. This results in a decrease of the DNA melting point and scission of the DNA strands (13,14).

MATERIALS AND METHODS

Between September 1969 and May 1970, 22 patients were accepted for treatment with bleomycin. All patients had had previous surgical treatment, radiotherapy, chemotherapy, or a combination of these, and demonstrated objectively progressive tumor growth at the time of entry to this study. All had become resistant to standard and accepted modalities of therapy. No attempt was made to select patients as to type of neoplasm, although some preference was given to differentiated epidermoid carcinoma in view of the Japanese reports (tables 1 and 2).

TABLE 1.—Phase I bleomycin study: patients treated

Total treated	22
Not evaluable	3
a. Contact lost	1
b. Early death	2
Evaluable	19
a. Biweekly therapy	9
b. Daily therapy	10

Initially, bleomycin, at a dose of 30 mg iv, was given twice weekly. The average single dose was 0.45 mg/kg ranging from 0.37 to 0.60 mg/kg. As experience was gained with the drug, several inpatients were treated with daily iv doses of 15 mg. The average single daily dose was 0.25 mg/kg, ranging from 0.19 to 0.30 mg/kg. The selection of dosage schedule was strictly a matter of convenience. Patients initially seen as inpatients were given daily treatment and those seen initially as outpatients were treated twice weekly. This may have resulted in a selection of more seriously ill patients for the daily treatment regimen. To evaluate drug tolerance, all patients were treated until some degree of toxicity occurred. Those patients showing tumor regression were continued or restarted on treatment until objective evidence of tumor progression was established, or until toxic effects, despite dosage adjustments, prohibited further treatment. If no tumor response was noted, therapy was stopped when toxicity was noted.

Tumor status[5] and performance status (Karnofsky) were established in all cases at

[5] Clinically or radiologically measurable tumor masses. A decrease in the product of two diameters of 25%–50% = +, 50%–90% = ++, and 90%–100% = +++.

TABLE 2.—Phase I bleomycin study: tumors treated

Head and neck carcinoma	3	Round cell sarcoma	1
Bronchus carcinoma	4	Vaginal carcinoma	1
Hodgkin's disease	2	Parotid carcinoma	1
Choriocarcinoma (male)	1	Reticulum cell sarcoma	1
Embryonal cell carcinoma	1	Undifferentiated abdominal carcinoma	1
Leiomyosarcoma	1	Epidermoid bladder carcinoma	1
Chondromyosarcoma	1		

the start of treatment and recorded at least weekly during the first weeks of therapy. Laboratory studies included white blood cell and platelet counts, and determinations of hematocrit, blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) levels, before, during, and after treatment. Radiologic controls were performed at least monthly and at the end of the study, or as indicated by the clinical course of the individual patient.

RESULTS

Of the 22 patients entered into the study, 19 were treated until some degree of toxicity resulted and their cases could be evaluated. Two patients died as a direct result of their tumor within the first week of treatment and could not be evaluated. One of these patients, who had pronounced local extension of a well-differentiated squamous cell carcinoma of the larynx, died on the third day of treatment with massive local hemorrhage. Since it was felt that the tumor was regressing in size at the time, this death may have been indirectly attributable to a good bleomycin effect. A third patient with pulmonary metastases from a locally recurrent cervical carcinoma was lost to followup after the first bleomycin injection. She died at home several weeks later.

TOXICITY

The toxic effects noted in the individual patients are shown in table 3 for the group treated biweekly and in table 4 for the group treated daily. Table 5 enumerates the various forms of toxicity recorded. It also gives the total number of patients manifesting each type of toxicity as well as the number of times each specific effect was the primary cause of discontinuing treatment in any one patient. There is some overlap in the cases with hematologic toxicity. Only one patient had isolated leukopenia without thrombocytopenia.

TABLE 3.—Patients treated with 30 mg of bleomycin biweekly

		Dosage			Toxicity				Results	
Patient No.	Age	Total dose (mg)	Mg/kg *	Mg/kg/day *	Skin reaction †	Fever †	Stomatitis †	Hematologic toxicity ‡	Objective §	Subjective ¶
1	42	480	6	0.12	++	+	+++	0	++	++
2	38	370	6.2	0.02	+++	+	+	++	++	+++
3	68	225	3.1	0.08	+	+++	0	+	0	+
4	46	255	3.6	0.10	++	0	0	0	0	+
5	36	90	1.4	0.15	0	+++	0	+++	P	0
6	30	750	15	0.13	+	+	0	0	0	+
7	49	195	2.6	0.10	++	++	+	0	0	0
8	46	390	7.1	0.15	++	0	0	0	P	P
9	53	285	3.7	0.10	++	+	++	0	P	+
Total					8/9	7/9	4/9	3/9	2/9	6/9

* Total doses received when therapy was initially stopped because of toxicity. Responders received further treatment at tolerable dosage levels until tumor progression occurred, or toxicity forced cessation of therapy, or both (*see* table 6).

† 0 = none; + = mild; ++ = moderate; +++ = severe.

‡ 0 = none; + = wbc of 3000–4000/mm³, or platelet count of 75,000–100,000/mm³, or both; ++ = wbc of 1500–3000/mm³, or platelet count of 25,000–75,000/mm³, or both; +++ = wbc < 1500/mm³, or platelet count < 25,000/mm³, or both.

§ + = decrease in the product of 2 diameters of clinically or radiologically measurable tumor masses of 25%–50%; ++ = decrease of 50%–90%; +++ = decrease of 90%–100%; P = progression.

¶ + = mild improvement; ++ = > 50% improvement; +++ = completely asymptomatic; P = progression.

TABLE 4.—Patients treated with 15 mg of bleomycin daily

Patient No.	Age	Dosage			Toxicity				Results	
		Total dose (mg)	Mg/kg *	Mg/kg/day *	Skin reaction †	Fever †	Stomatitis †	Hematologic toxicity ‡	Objective §	Subjective ¶
10	25	570	11	0.18	+	+	+	0	++	++
11	75	90	1.7	0.28	+	0	0	++	+++	++
12	32	180	2.6	0.09	++	++	+	++	++**	+++
13	75	195	3.4	0.26	++	0	++	0	+††	+††
14	50	150	2.1	0.18	0	0	0	0	0	+‡‡
15	67	315	3.9	0.15	++	+	+	0	0	+
16	78	375	4.9	0.08	++	0	0	0	0	+
17	64	75	1.2	0.19	0	0	0	+	0	0
18	49	240	4.8	0.21	++	++	0	0	0	+
19	71	225	3.0	0.17	++	0	+	0	0	+
Total					8/10	4/10	5/10	3/10	4/10	8/10

* Total dose received when therapy was initially stopped because of toxicity. Responders received further treatment at tolerable dosage levels until tumor progression occurred, or toxicity forced cessation of therapy, 1500–3000/mm³, or platelet count of 25,000–75,000/mm³, or both; +++ = wbc < 1500/mm³, or platelet count or both (*see* table 6).

† 0 = none; + = mild; ++ = moderate; +++ = severe.

‡ 0 = none; + = wbc of 3000–4000/mm³, or platelet count of 75,000–100,000/mm³, or both; ++ = wbc of 1500–3000/mm³, or platelet count of 25,000–75,000/mm³, or both; +++ = wbc < 1500/mm³, or platelet count < 25,000/mm³, or both.

§ + = decrease in the product of 2 diameters of clinically or radiologically measurable tumor masses of 25%–50%; ++ = decrease of 50%–90%; +++ = decrease of 90%–100%; P = progression.

¶ + = mild improvement; ++ = > 50% improvement; +++ = completely asymptomatic; P = progression.

** Massive hemorrhagic cystitis.

†† Simultaneous radiotherapy.

‡‡ Taste distortion, anorexia.

Skin Reaction

Skin reaction was the most frequently encountered toxic effect in both dosage regimens (16 to 19 patients). It consisted of an initial thickening of the epidermis, most commonly seen at the finger tips, palms, soles, and elbows. At the onset, patients often complained of mild associated hypoesthesia of the affected skin areas. With progression, local hyperesthesia was often encountered and the lesions became painful. In the more severe forms of toxicity, bullous lesions with subsequent peeling of the skin were seen. This often occurred when the affected areas were not protected from further external pressure or minute trauma. Permanent defects were not encountered. Frequently, severe pigmentation of the skin folds, scars, or other pressure areas was seen. This pigmentation was reminiscent of Addison's disease. Aside from the cosmetic effect, it was of no further consequence and never caused discontinuation of therapy.

Varying degrees of alopecia also occurred in 12 of the 19 patients. Again, it was primarily a cosmetic problem and treatment was never discontinued because of it. In all cases of alopecia there was regrowth of the hair after therapy was stopped, provided the patient survived long enough.

Fever

Fever was the second most commonly encountered toxic effect; it was noted in 11 of the 19 patients. It almost always occurred several hours after bleomycin injection and lasted from 8 to 12 hours, usually decreasing in frequency

TABLE 5.—Bleomycin toxicity

Toxic effect	Total No. of occurrences of toxic effect	No. of times therapy was discontinued because of toxicity *
Skin reaction		
Pigmentation	13/19	0
Thickening	16/19	11/19
Stomatitis	9/19	3/19
Alopecia	12/19	0
Fever and chills	11/19	(2) †
Nausea and vomiting	5/19	0
Fall in hematocrit level	3/19	0
Leukopenia	3/19	3/19
Thrombocytopenia	5/19	5/19
Rise in BUN value	(1)‡/19	0
Rise in alkaline phosphatase level	2/19	0
Rise in SGOT/SGPT levels	3/19	0
Fall in serum protein level	2/9	0

* See text for overlap of hematologic toxicity.
† Therapy would have had to be discontinued for this toxic effect in these 2 patients if they had not also had hematologic toxic effects which forced the end of treatment.
‡ One isolated transient elevation in a single patient.

and severity or ceasing completely as the treatment continued. The elevated temperatures always responded to salicylate therapy. Although this form of toxicity was never the primary reason for stopping therapy, it easily could have stopped treatment in two patients who had shaking chills, profuse diaphoresis, and temperatures to 39 C despite dosage reduction, if they had not also had hematologic toxicity which caused discontinuance of treatment. In contrast to these two cases, most patients with hyperthermic reactions were remarkably free of any associated subjective discomfort. It often appeared that those in the immediate environment (nurses, physicians, and family) were more adversely affected than the patient.

Stomatitis

Stomatitis was a relatively frequent form of toxicity. It was noted in nine of the 19 patients and was the primary cause of therapy discontinuation in three. Patients with poor dental care (jagged or decayed teeth) or with ill-fitting dental prostheses were most commonly affected. Proper prophylaxis such as the removal of improperly adjusted dentures and the avoidance of pressure areas (similar to the skin toxicity) aided in the rapid healing of the mucosal lesions and often permitted therapy to be continued.

Hematologic Toxicity

Hematologic toxicity was noted in six of the 19 patients and was the primary reason for discontinuing therapy in these six patients. Mild leukopenia (with a fall in wbc count from 5400/mm^3 to 3300/mm^3) developed in one patient; no thrombocytopenia was noted. There were two cases of leukopenia associated with thrombocytopenia (one to 644/mm^3 and another to 1100/mm^3), both with initial values over 4000/mm^3. In three patients, isolated thrombocytopenia (54,000/mm^3 to 78,000/mm^3) without leukopenia was noted. With one exception, all cases of marrow toxicity could be accounted for by decreased marrow reserves due to diffuse marrow invasion by tumor cells, intensive long-term previous radiotherapy, or chemotherapy, or a combination of these. The marrow depression was quickly reversible in all cases after bleomycin was discontinued. There were no toxic deaths.

A fall of 6% in the hematocrit level was noted in three cases. In one patient with stage IV Hodgkin's disease, this was accompanied by leukopenia and thrombocytopenia; in the other two cases it was an isolated hematologic finding and could be explained by blood loss in one (ulcerating vaginal tumor), and by markedly progressive cachexia and malnutrition in another (head and neck tumor).

Gastrointestinal Toxicity

Nausea and vomiting developed in five of the 19 patients during treatment with bleomycin. It was never severe enough to discontinue therapy, and was usually well controlled with antiemetics. Another two patients had moderate anorexia and complained of a persistent bad

taste in the mouth. Diarrhea was noted on only one occasion and its relation to bleomycin therapy is questionable.

Blood Chemistry Tests

Renal function as indicated by blood urea nitrogen (BUN) level was not affected. Only one patient had a transient rise in the BUN level to 22 mg/100 ml. This isolated elevation returned to normal despite continued daily bleomycin therapy.

Pathologic *alkaline phosphatase* levels developed in two patients. In both, there was a strong suspicion of liver metastases (one embryonal cell carcinoma and one reticulum cell sarcoma). One patient who had high values initially, showed a further slight rise during therapy. Three other patients with initially elevated levels returned to normal during bleomycin treatment.

SGOT and SGPT rises to pathologic levels were noted in three patients, but these rises never caused discontinuation of treatment. One patient who had elevated transaminase values at the start of therapy was suspected of having liver metastases, and the values varied widely during treatment. The other two patients had only slight isolated elevations which returned to normal despite continued therapy with bleomycin. In no case were values greater than 55 units. In four other patients, initially elevated transaminase levels returned to normal during treatment.

A fall in the *serum protein* value was observed in two of nine patients. In both, the drops were minimal (0.7 and 1.0 g/100 ml) and remained within normal ranges (8.3 and 7.5 g/100 ml). In another patient a slight rise was noted.

Other Toxic Effects

One patient had repeated severe hematuria and cystitis after bleomycin administration. Cystoscopy on two occasions showed hemorrhagic cystitis. Although tumor invasion of the bladder was suspected, it could not be proved either at cystoscopy or at autopsy.

Interstitial pneumonia occurring after larger bleomycin doses, as reported especially in older patients (15), was conscientiously sought but was never encountered either clinically or radiologically in any of our patients. Chest X-ray films were made every 2–4 weeks for patients treated for more than 2 weeks or receiving more than 200 mg bleomycin. For patients with pulmonary symptoms at the start of treatment, roentgenograms were made more frequently. Pulmonary function studies were performed before (and repeated during) bleomycin therapy in three patients with initial pretreatment dyspnea or asthma. Of the 19 patients studied, seven died of progressive tumor within 2 months after bleomycin was stopped. Three of these patients were autopsied and no interstitial pneumonia was found. In two others, chest X-ray films made within 2 weeks before death showed no pneumonia. Four of our patients were over 70 years of age and received total doses of 90–375 mg bleomycin (1.7–4.9 mg/kg) within 6–90 days.

TUMOR RESULTS

Definite tumor responses were noted in six (31%) of the 19 patients treated until toxicity occurred (table 6). One patient with a large infiltrating and ulcerating vulval carcinoma had only a 25%–50% tumor regression. This regression was, however, sufficient to allow an attempt at radical surgical treatment which had been impossible before combined bleomycin therapy and radiotherapy. Previous radiotherapy alone produced no significant tumor regression. Four patients had satisfactory tumor regression of greater than 50%. One patient with a disseminated round cell sarcoma showed complete tumor regression. This patient had recurrent leukopenia and thrombocytopenia during bleomycin therapy, therefore, treatment was limited, and she died of tumor progression 45 days after the start of therapy.

The patient with Hodgkin's disease showed tumor progression in addition to moderately severe skin toxicity after 283 days of treatment. Therapy was changed to CCNU,[6] and he is still alive.

Severe hemorrhagic cystitis developed after

[6] CCNU: NSC–79037; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

TABLE 6.—Tumor responses after bleomycin treatment

Diagnosis	Age	Total dose/ toxic dose (mg)	Dose given until toxicity occurs *		Treatment schedule	Results		Therapy duration (days)	Survival time from start of therapy (days)
			Mg/kg	Mg/kg/ day		Objective †	Subjective ‡		
Round cell sarcoma	75	165/90	1.7	0.28	Daily	+++	++	21	45
Hodgkin's disease	38	370/370	6.2	0.02	Biweekly	++	+++	283	Therapy change, still alive
Undifferentiated abdominal carcinoma	32	240/180	2.6	0.09	Daily	++	+++	56	80
Reticulum cell carcinoma	42	480/480	6.0	0.12	Biweekly	++	++	52	86
Chorio-carcinoma	25	570/570	11.0	0.18	Daily	++	++	60	75
Vaginal carcinoma	75	195/195	3.4	0.28	Daily	+	+	Surgical treatment §	—

* Mg/kg and mg/kg/day doses represent dose levels at which therapy was stopped because of toxicity. In 4 patients these levels were identical to the total dose given. In 2 patients treatment was resumed at reduced dosage after recovery from toxicity in an attempt to maintain remission as long as possible.

† + = decrease in the product of 2 diameters of clinically or radiologically measurable tumor masses of 25%–50%; ++ = decrease of 50%–90%; +++ = decrease of 90%–100%; P = progression.

‡ + = mild improvement; ++ = > 50% improvement; +++ = completely asymptomatic; P = progression.

§ This patient underwent radical surgical treatment immediately after combined bleomycin therapy and radiotherapy.

56 days of treatment in the patient with undifferentiated abdominal carcinoma. Bleomycin therapy was discontinued because of this despite a continuing tumor remission.

Severe skin toxicity after 52 days of therapy developed in the patient with reticulum cell sarcoma, and bleomycin treatment was stopped. The tumor recurred and the patient died before the toxic effects subsided enough to allow reinstitution of treatment.

Brain metastases developed in the patient with choriocarcinoma during bleomycin therapy. Treatment was stopped after 60 days, and he died 2 weeks later.

In nine patients, there was no objective evidence of tumor response during or after treatment. Four patients showed definite tumor progression despite therapy until toxicity occurred.

Subjective responses, as judged by improvement in performance status or relief from pain or both, were noted in all patients with objective tumor regression. Subjective improvement was also noted in six patients whose tumors showed no change, and in one patient with tumor progression. Weight increases were recorded in five patients, three of whom had tumor regression. Six patients had weight losses, including three who had tumor progression. Eight patients had no weight change.

DISCUSSION

The toxic effects of bleomycin in the dosage schedules used in this study were tolerable. Toxicity was always reversible after discontinuation of the drug. With the exception of fever, no significant differences in the two dosage schedules regarding development or severity of toxicity were noted. Fever was noted more frequently in the 30-mg biweekly group than in the 15-mg daily group. This was

probably due to the larger single dose in the former group, since previous reports have shown this manifestation to be decreased or eliminated by reduction of the single dose (16, 17). Our experience confirmed this.

The appearance of leukopenia or thrombocytopenia or both with bleomycin therapy was surprising. Previous reports of clinical trials have uniformly confirmed the lack of hematopoietic toxicity (15,17). The following case reports describe the occurrence of hematologic toxicity in six patients.

Patient No. 2.—A 38-year-old man with Hodgkin's disease (lymphogranuloma) of 4½ years' duration was treated biweekly with 30 mg of bleomycin. Previously, he had had long-term intensive chemotherapy as well as radiotherapy for a gastric tumor. On Day 23, treatment was stopped because of a platelet fall from 254,000/mm³ to 54,000/mm³ and painful skin toxicity. Leukopenia never occurred. The patient had good objective and subjective tumor response, and therapy with 15 mg weekly was started 3 weeks later when the skin lesions had healed. He remained in remission for 283 days and received a total dose of 370 mg without any further hematologic toxicity.

Patient No. 3.—A 68-year-old man with bronchial epidermoid carcinoma had had no previous radiotherapy or chemotherapy. Thirty mg of bleomycin was given biweekly. On Day 40 a platelet drop from 219,000/mm³ to 78,000/mm³ was noted. The thrombocytopenia abated rapidly after bleomycin was discontinued.

Patient No. 5.—A 36-year-old man with Hodgkin's sarcoma of 3 years' duration was given 30 mg of bleomycin twice weekly. Previous treatment consisted of initial radical radiotherapy and subsequent intensive cytostatic therapy. Bone marrow examinations were not conclusive, but the regular appearance of immature granulocytes and nucleated red blood cells in the peripheral blood made the probability of marrow infiltration with tumor very high. In view of this, treatment was continued despite an initial fall of the wbc from 4700/mm³ to 640/mm³ and a platelet drop from 105,000/mm³ to 30,000/mm³ 6 days after the start of therapy. Three days later the wbc had again risen to 2300/mm³. The patient died on the 11th day of therapy due to tumor progression.

Patient No. 11.—A 75-year-old woman with disseminated lesions of a round cell sarcoma was treated with daily 15-mg doses of bleomycin. She had received intensive chemotherapy for 1 year as well as radiotherapy to the lumbar spine before bleomycin therapy. There was also diffuse marrow invasion with tumor cells present. After 6 days of treatment, the wbc fell from 4300/mm³ to 2600/mm³ and the platelets fell from 251,000/mm³ to 83,000/mm³. Recovery was rapid after discontinuing therapy.

Patient No. 12.—A 32-year-old man was treated for 3½ years with intensive chemotherapy for disseminated undifferentiated abdominal carcinoma. He was leukopenic (wbc, 2500/mm³) at the start of bleomycin therapy (15 mg daily). A further drop in the wbc was not noted; in fact, the counts rose slightly. However, the platelets dropped from 160,000/mm³ to 60,000/mm³ by Day 29 of therapy. Severe hemorrhagic cystitis also developed in this patient during therapy. Unfortunately, fibrinogen levels were not determined and a consumption coagulopathy could not be excluded.

Patient No. 17.—A 64-year-old woman with local extension of a trabecular polymorph cell carcinoma of the parotid gland was treated with 15 mg of bleomycin daily. The tumor was present for over 5 years, and the patient had received repeated surgical treatment and many courses of radiotherapy. The wbc dropped from 5400/mm³ to 3700/mm³ on Day 6. Treatment was stopped, and the count fell to 3300/mm³ by Day 10. On Day 17 the wbc was 6000/mm³. No fall in hematocrit level or platelet count was noted.

With the exception of patient No. 3, all these patients had either extensive radiotherapy or intensive chemotherapy over longer periods of time. In two cases, bone marrow infiltration was either proved or strongly suspected. In no case was the marrow depression life threatening, irreversible, or long lasting.

Although nine of the 19 patients had squamous cell carcinoma, only one of these showed a slight tumor regression, and this was achieved with combined radiotherapy. The number of

cases is admittedly small, but it is remarkable that this allegedly sensitive histologic tumor type did not show a greater response rate. On the other hand, the patients with malignant lymphoma responded well. The tumor responses noted in the two testicular neoplasms treated (one definite regression and one stabilization with subjective improvement of a previously rapidly progressive tumor) were very satisfying.

The number of responders in each dosage group is too small to give a statistically valid preference. It should be noted, however, that the patient with pulmonary metastases of a testicular choriocarcinoma showed a definite tumor regression with 15 mg daily. This response could not be subsequently maintained on the biweekly schedule.

The total dose and the milligram per kilogram dose could not be correlated with tumor response. These doses were obviously higher in the tumor responders, since therapy was continued in these patients at a tolerable dose until the remission ended. Also, the dosage calculated on a milligram per kilogram per day basis produced no great differences between responders and nonresponders. It served only to demonstrate a relatively nontoxic dosage range for long- or short-term therapy.

CONCLUSIONS

Bleomycin given at a dose of 30 mg iv twice weekly or 15 mg iv daily is relatively well tolerated. Toxicity was never lethal and always reversible when treatment was stopped. Special caution is recommended when patients with depleted bone marrow reserves, due to either marrow infiltration with tumor or to previous intensive or long-term radiotherapy or chemotherapy, are treated. Leukopenia, thrombocytopenia, or both may be encountered in such cases. The most frequent forms of toxicity noted were skin thickening and pain, fever, and stomatitis. These could have become severe if therapy had not been discontinued, but they were always reversible. Results with squamous cell carcinoma were disappointing, but the number of patients treated was relatively small. Results with malignant lymphomas and testicular tumors seemed promising. Relatively resistant tumor forms may respond better to a 15-mg daily schedule than to a 30-mg biweekly schedule. Certainly, further studies in these areas, possibly using combinations with other cytostatic agents, are indicated.

REFERENCES

1. UMEZAWA, H., MAEDA, K., TAKEUCHI, T., ET AL. New antibiotics, bleomycin A and B. J Antibiot 19:200–209, 1966.
2. MAEDA, K., KOSAKA, H., YAGISHITA, K., ET AL. A new antibiotic, phleomycin. J Antibiot 9:82–85, 1956.
3. ISHIZUKA, M., TAKAYAMA, H., TAKEUCHI, T., ET AL. Studies on antitumor activity, antimicrobial activity and toxicity of phleomycin. J Antibiot 19:260–271, 1966.
4. ISHIZUKA, M., TAKAYAMA, H., TAKEUCHI, T., ET AL. Activity and toxicity of bleomycin. J Antibiot 20: 15–24, 1967.
5. UMEZAWA, H., SUHARA, Y., TAKITA, T., ET AL. Purification of bleomycins. J Antibiot 19:210–215, 1966.
6. TAKITA, T., MURAOKA, Y., MAEDA, K., ET AL. Chemical studies on bleomycin. I. The acid hydrolysis products of bleomycin A2. J Antibiot 21:79–80, 1968.
7. KOYAMA, G., NAKAMURA, H., MURAOKA, Y., ET AL. The chemistry of bleomycin. II. The molecular and crystal structure of a sulfur-containing chromophoric amino acid. Tetrahedron Lett 44:4635–4638, 1968.
8. TAKITA, T., MAEDA, K., UMEZAWA, H., ET AL. Chemistry of bleomycin. III. The sugar moieties of bleomycin A2. J Antibiot 22:237–239, 1969.
9. ICHIKAWA, T., MATSUDA, A., MIYAMOTO, K., ET AL. Biological studies on bleomycin A. J Antibiot 20: 149–155, 1967.
10. UMEZAWA, H. Fundamental studies on bleomycin. Institute of Microbial Chemistry, Department of Antibiotics. National Institute of Health, Institute of Applied Microbiology. University of Tokyo, 1970.
11. SUZUKI, H., NAGAI, K., YAMAKI, H., ET AL. Mechanism of action of bleomycin. Studies with the growing culture of bacterial and tumor cells. J Antibiot 21:379–386, 1968.
12. KUNIMOTO, T., HORI, M., and UMEZAWA, H. Modes of action of phleomycin, bleomycin and formycin on HeLa S3 cells in synchronized culture. J Antibiot 20:277–278, 1967.
13. NAGAI, K., SUZUKI, H., TANAKA, N., ET AL. Decrease of melting temperature and single strand scission of DNA by bleomycin in the presence of 2-mercaptoethanol. J Antibiot 22:569–573, 1969.
14. NAGAI, K., SUZUKI, H., TANAKA, N., ET AL. Decrease of melting temperature and single strand scission of DNA by bleomycin in the presence of hydrogen peroxide. J Antibiot 22:624–628, 1969.
15. CLINICAL SCREENING COOPERATIVE GROUP OF THE EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER. Study of the clinical efficiency of bleomycin in human cancer. Br Med J 2 (5710): 643–645, 1970.
16. ICHIKAWA, T., NAKANO, I., and HIROKAWA, I. Bleomycin treatment of the tumors of penis and scrotum. J Urol 102:699–707, 1969.
17. ICHIKAWA, T. The clinical effect of bleomycin against squamous cell carcinoma and further developments. Proc 6th Int Congr Chemother, Tokyo, 1970, vol 2, pp 288–290.

Phase II Study of Bleomycin (NSC-125066) Therapy for Large Bowel Cancer [1, 2, 3]

C. G. Moertel, P. J. Arena, A. J. Schutt, R. J. Reitemeier, and R. G. Hahn [4]

SUMMARY

Fifteen patients with metastatic large bowel cancer were treated with bleomycin given intravenously at a daily dose of 20 mg/m² to a maximum total dose of 280 mg. Toxic reactions included nausea, vomiting, chills, fever, dermatitis, stomatitis, alopecia, and occasional mild thrombocytopenia. A probable pulmonary toxic reaction occurred in one patient treated with a total dose of 252 mg. The patient died of acute renal insufficiency 11 days after completion of therapy. Objective evidence of tumor regression was not noted in any of the 15 patients.

[Cancer Chemother Rep 56:207–210, 1972]

Bleomycin is an antibiotic made up of a mixture of water-soluble polypeptides derived from *Streptomyces verticillis*. It inhibits the incorporation of thymine into DNA and has produced inhibition of Ehrlich carcinoma and Sarcoma 180. After systemic administration it is selectively concentrated in the skin, lungs, and kidneys (1).

Clinically, bleomycin has attracted great interest, particularly in the treatment of epidermoid types of carcinoma. More recent experience, however, has indicated a broader range of drug activity with objective tumor regressions recorded for patients with lymphoma, testicular carcinoma, melanoma, and salivary gland adenocarcinoma (2–6). This study was undertaken as a phase II screen for possible clinical activity in adenocarcinoma of the large bowel.

MATERIALS AND METHODS

Fifteen patients who had pathologically confirmed metastatic adenocarcinoma of the large bowel were selected for study. Seven of these had had no previous chemotherapy, and eight had had treatment with 5-fluorouracil (NSC-19893) or camptothecin (NSC-100880). Three patients had had previous focal radiation therapy. No patient had received either radiation or chemotherapy within 1 month preceding the start of bleomycin treatment. All were ambulatory outpatients, and all were maintaining a reasonable nutritional intake. Each patient had a measurable area of known cancer to serve as an objective indicator of response to therapy.

Bleomycin was reconstituted in 5% glucose solution and administered by intravenous injection at a daily dose of 20 mg/m². Treatment was continued until significant mucocutaneous reactions occurred or until a total dose of 280 mg had been administered. Laboratory assessments of renal and hepatic functions and hematologic status, as well as chest roentgenogram, were obtained on the day before therapy, the day after therapy, and 1 month after completion of treatment. Leukocyte counts were ob-

[1] Received Sept 9, 1971; accepted Nov 1, 1971.
[2] Supported by contract NIH–70–2066 from Chemotherapy, National Cancer Institute (NCI), National Institutes of Health.
[3] Bleomycin was supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, NCI.
[4] Division of Gastroenterology and Internal Medicine (Drs. Moertel, Reitemeier, and Schutt), Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Graduate School (Dr. Arena), Mayo Clinic and Mayo Foundation, Rochester, Minn.

tained twice weekly and platelet counts once weekly after treatment. Therapeutic results were assessed 1 month after completion of therapy.

RESULTS

Toxic Reactions

Thirteen patients received the entire planned dose of 280 mg. For two patients, treatment was discontinued after total doses of 165 and 252 mg had been administered due to the development of moderately severe stomatitis and dermatitis.

The occurrence of toxic reactions in our 15 patients is recorded in table 1. Nausea was an almost universal symptom and vomiting was frequent. Afternoon fever, frequently accompanied by shaking chills, was also common, particularly early in the course of treatment. Highest temperatures ranged between 100 and 104.8 F. Cutaneous reactions, the most distinctive feature of bleomycin toxicity in this study, included erythematous and painful digital swelling, vesicular and desquamating palmar eruptions, and eczematoid dermatitis of the elbows and dorsum of the hands followed by pigmentation. Stomatitis with focal ulcerations was observed in approximately one half of our patients, frequently beginning in areas of denture pressure points. Varying degrees of alopecia were noted also in most patients. Less common mucous membrane reactions included conjunctivitis, vaginitis, and esophagitis.

A significant hypotensive effect during treatment was thought to be demonstrated in seven patients by reductions in systolic blood pressures of from 40 to 60 mm Hg or reductions in diastolic blood pressures of from 20 to 40 mm Hg. These changes were usually manifest by the third treatment day and could not be clearly attributed to other drugs the patients might have been receiving, eg, analgesics or antiemetics. There were no associated symptoms in this group of patients; however, in one subsequently followed patient receiving bleomycin in combination with radiation therapy, we observed a drop in blood pressure from a pretreatment level of 148/92 mm Hg to 86/46 mm Hg on the eighth treatment day. The drop was associated with severe postural vertigo. This patient was not receiving any concomitant drug therapy.

In contrast to earlier reports, we did find evidence of hematologic toxicity manifested by significant falls in hemoglobin levels in two patients and by relatively mild thrombocytopenia in five patients reaching nadirs of from 60,000/mm³ to 160,000/mm³ 15–21 days after the beginning of therapy.

One patient died as a probable result of bleomycin toxicity. This was a 60-year-old man who had nausea, vomiting, chills, and fever to 103.6 F during treatment. Therapy was discontinued after administration of 252 mg (120 mg/m² over 6 days) because of his moderately severe stomatitis. On Day 6, he noted painless hematuria; urinalysis revealed, in addition, proteinuria and scattered hyaline, waxy, granular, leukocyte, and red blood cell casts. On Day 7, the serum creatinine value had risen from 1.05 to 7.8 mg/100 ml. A chest roentgenogram showed small poorly defined infiltrates at both lung bases. By Day 15, the hemoglobin level had fallen from 11 to 6.6 g/100 ml and the platelet count from 495,000/mm³ to 60,000/mm³. The patient became oliguric, and on Day 16 his blood urea nitrogen level was 135 mg/100 ml. On this day, a chest roentgenogram showed diffuse and severe pulmonary infiltration (fig 1). He died on the 17th day after the start of therapy (11 days after the last dose). Necropsy examination was not permitted. This patient had significant hematologic and probably pulmonary toxic reactions. His death was attributed clinically to acute renal insufficiency.

TABLE 1.—Toxic reactions to bleomycin therapy in 15 patients

Toxic reactions	Patients
Nausea	14
Vomiting	10
Fever	13
Shaking chills	10
Dermatitis	13
Alopecia	11
Stomatitis	8
Conjunctivitis	2
Vaginitis	1
Esophagitis	1
Pulmonary infiltration	1
Renal insufficiency	1
Thrombocytopenia (<200,000/mm³)	5
Anemia (fall in Hgb level >3 g/100 ml)	2

Although the possibility of a coincident acute ureteral obstruction could not be excluded without necropsy, it is probable that this case is an example of fatal bleomycin renal toxicity.

Therapeutic Results

One month after completion of therapy (5–6 weeks after start), three of our 15 patients claimed partial symptomatic improvement, and one patient, who was jaundiced before therapy, had a transient fall in serum bilirubin level. Objective measurements of indicator lesions showed that seven patients had remained objectively unchanged and seven patients had had progression of their cancer. One patient had died, presumably as a result of drug toxicity.

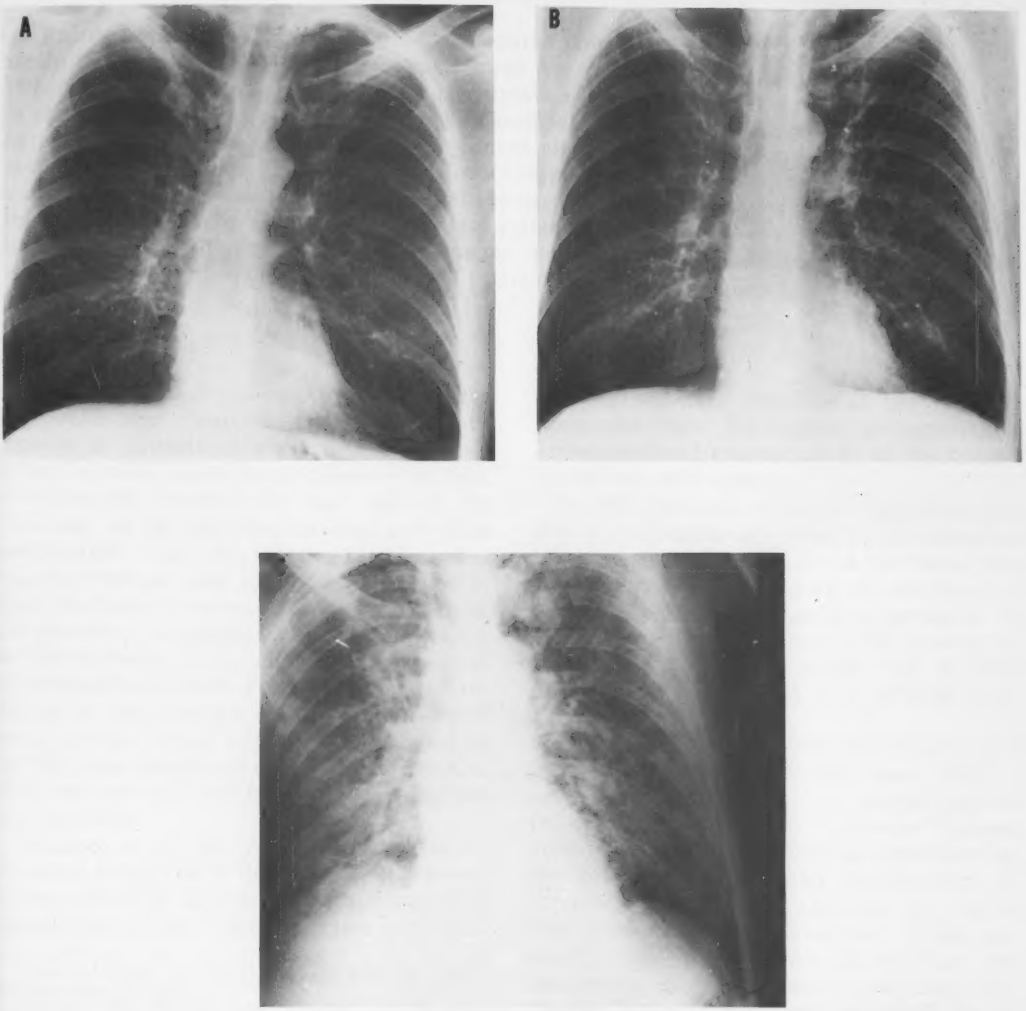


FIGURE 1.—*A*: Pretreatment chest roentgenogram. *B*: Day 7—early infiltrates in midportion of both lower lobes. *C*: Day 17—diffuse interstitial and alveolar pulmonary infiltration.

REFERENCES

1. UMEZAWA, H., ISHIZUKA, M., MAEDA, K., ET AL. Studies on bleomycin. Cancer 20:891–895, 1967.
2. BONADONNA, G., DELENA, M., BARTOLI, C., ET AL. Clinical trial with bleomycin in lymphomas and solid tumors. Proc Am Assoc Cancer Res 12:11, 1971.
3. Clinical Screening Co-op Group of the European Organization for Research on the Treatment of Cancer. Study of the clinical efficiency of bleomycin in human cancer. Br Med J 2:643–645, 1970.
4. LUCE, J. K., FREIREICH, E. J, LUNA, M., ET AL. Clinical trials with daily IV bleomycin. Proc Am Assoc Cancer Res 12:83, 1971.
5. SELAWRY, O. S., OHNUMA, T., and SHEDD, D. P. Bleomycin: tolerance of cancer patients to twice weekly dosage. Proc Am Assoc Cancer Res 12:70, 1971.
6. YAGODA, A., KRAKOFF, I., LAMONTE, C., ET AL. Clinical trial of bleomycin. Proc Am Assoc Cancer Res 12:37, 1971.

Clinical Experiences With the Streptococcal Anticancer Preparation, OK-432 (NSC-B116209) [1,2]

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SUMMARY

The new anticancer preparation, OK-432, prepared from a low virulent strain (Su) of *Streptococcus hemolyticus* (Group A), was given to 43 patients with advanced cancer, and a discernible effect was noted in 14 (32.6%) of these patients. Comparatively beneficial results were noted in patients with stomach and breast cancer, and some effect occurred in the patients that had not previously responded to the available anticancer agents. It appears that the carcinostatic effect of this agent is primarily due to its cancerocidal action and partly to its host-mediated activity. OK-432 is a unique carcinostatic drug with a new mechanism of action which is of interest to clinicians.
[Cancer Chemother Rep 56:211–220, 1972]

Since Busch first reported in 1868 (1) the regression and disappearance of malignant tumors in patients infected with erysipelas, several similar reports have appeared. In 1882, *Streptococcus erysipelatis* was isolated by Fehleisen as the pathogen causing erysipelas and, in 1891, Coley (2), utilizing the same clinical observations used by Busch, attempted to treat malignant tumors with preparations of *Streptococcus erysipelatis*. Erysipelas was a serious disease at that time and this treatment often resulted in death. For this reason, culture filtrate or heated-killed culture free of viable cocci, such as Coley's mixed toxin, was used in animal experiments and clinical trials, but constant potency was hard to obtain and the effect was uncertain.

Okamoto et al (3) reported that a short period of incubation of the *Streptococcus hemolyticus* cells with Ehrlich ascites tumor cells in suspension resulted in the production of streptolysin S, and that injection of the resulting suspension into the mouse peritoneum no longer produced ascites tumors in the animals (in vitro-in vivo system).

In 1966, Okamoto et al (3) discovered that the in vivo anticancer effect of *Streptococcus hemolyticus* on Ehrlich ascites carcinoma was greatly increased by treating the streptococcal cells with penicillin and heating further at 45 C. The treated cells lost the ability to cause infection and to produce streptolysin S. This preparation was referred to as PCB-45 (OK-431).

Sakurai et al (4) reported that OK-431 and its lyophilized form, OK-432, were effective against various kinds of rat ascites tumors. OK-432 fails to grow in blood-agar medium after inactivation of the penicillin contained in the preparation (4), and proliferation of *Streptococcus* in vivo has also been observed to stop after various examinations.[4] Acute and subacute toxicity tests with OK-432 were carried out in mice, rats, rabbits, and dogs (4). Chronic toxicity tests were performed also in

[1] Received June 14, 1971; revised Nov 16, 1971; accepted Dec 15, 1971.
[2] OK-431 and OK-432 were supplied by Research Laboratories, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.
[3] Medical Clinic, Cancer Institute Hospital, Tokyo, Japan.

[4] Akiba, T., Ogawa, H., and Takagaki, Y. Personal communication.

rabbits and dogs.[4] In toxicity tests, pyrexia and leukocytosis were observed, but no changes in bone marrow were noted histologically (4). Thus, OK-432 was considered safe, and the trial use of the agent was started in February 1968 in patients with advanced cancer. This paper reports these results, together with some future problems.

MATERIALS AND METHODS

Materials

A low virulent strain, Su, of *Streptococcus hemolyticus* (Group A) was cultivated in 3% yeast extract medium at 37 C for 20 hours. The cells were collected and washed, and then resuspended in Bernheimer's basal medium (5) containing penicillin G (2.7×10^4 units/ml). After heating at 37 C for 20 minutes the cell suspension was further heated at 45 C for 30 minutes. This preparation is called OK-431 and its lyophilized preparation is called OK-432.

Initially, OK-431 was given to patients, but more recently OK-432 has been used for convenience, because the antitumor activity of OK-431 is relatively unstable and lost in a short time, while that of OK-432 is maintained much longer even at room temperatures.

The doses are expressed in KE (Klinische Einheit) units, with 10 KE corresponding to 1 mg of dried cell weight. A solution of 10 KE dissolved in 1 ml of physiologic saline solution produces a suspension containing approximately 10^9 cells.

Other antitumor agents used in previous treatments were thioTEPA, uracil mustard, chromomycin A_3, SPI-77, cyclophosphamide, 5-fluorouracil, mitomycin C, vinca alkaloids, bleomycin, NSC-123109 (T-39, a derivative of mitomycin C), NSC-84641 (No. 838), and L-asparaginase.[5,6]

[5] Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

[6] ThioTEPA and 5-fluorouracil: Sumitomo Chemical Co., Ltd., Osaka, Japan. Uracil mustard, mitomycin C, NSC-123109, and L-asparaginase: Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Chromomycin A_3: Takeda Chemical Industries, Ltd., Osaka, Japan. SPI-77: Sandoz Pharmaceutical Co., Ltd., Tokyo, Japan. Cyclophosphamide and the vinca alkaloids: Shionogi & Co., Ltd., Osaka, Japan. Bleomycin: Nippon Kayaku Co., Ltd., Tokyo, Japan. NSC-84641: Cancer Institute, Tokyo, Japan.

TABLE 1.—Number of patients treated with OK-432

Tumor types	No. of patients	No. of improvements
Carcinoma		
Stomach	23	8
Breast	4	3
Gall bladder	5	1
Lung	2	1
Rectum	2	0
Brain	1	0
Reticulosarcoma	3	1
Seminoma	1	0
Myeloid leukemia	1	0
Malignant melanoma	1	0
Totals	43	14 (32.6%)

Patients

As shown in table 1, there were 43 patients with incurable advanced cancer, including patients with recurrent disease in whom radiation therapy or other medication or both were ineffective. Some of the patients were in the advanced stages of disease and were severely debilitated.

Methods

OK-432 is a type of bacterial preparation different from any of the existing anticancer agents. At the time of our study, it had never been used for systemic clinical trial so that the method of administration, dose, and administration interval had not been established. For that reason, extensive trials were performed based on toxicologic data and antitumor effect in animals, and the chief methods of administration used in the present study were as follows:

(*a*) Local administration into the tumor: single administration of 5–10 KE daily or once every few days.

(*b*) Subcutaneous: 5–10 KE once every 4–5 days.

(*c*) Intracavitary: same as (*b*).

(*d*) Intravenous: initially 1 KE, then 2 KE, then 3 KE, once every 4–5 days.

The administration of OK-432 was started 1–2 months after the end of the most recent treatment.

The route of administration and the total dose of OK-432 administered are given in table

TABLE 2.—Effect of OK–432 related to method of administration and total dose

Total dose (KE)	No. of improvements/No. of patients treated				
	Route of administration*				
	it	sc	ic	iv	Total
≦10	0/0	0/3	0/2	3/7	3/12
11–30	0/1	0/2	0/0	5/12	5/15
31–50	2/4	0/0	1/1	0/0	3/5
51–100	0/5	0/0	3/4	0/0	3/9
≧101	0/1	0/1	0/0	0/0	0/2
Totals	2/11	0/6	4/7	8/19	14/43

*It = intratumoral; sc = subcutaneous; ic = intracavitary; iv = intravenous.

2. Since no serious side effects such as leukopenia or bone marrow injury were observed clinically after continued treatment, the general condition of each patient became the determining factor for the total amount to be administered to that patient. Iv injections were given in very small doses so that the total dose was always low, and the injections were generally given by mixing OK-432 in 500 ml of 5% glucose solution and administering by drip infusion over a 2-hour period.

During the initial period of the clinical experiments, a small group of patients received the earlier preparation (OK-431).

It was determined that a patient had objective improvement when the tumor (primary or metastasized foci) was reduced to less than 75% of the original size.

RESULTS

The results of treatment are showr in tables 1, 2, and 3. Objective improvement was clearly noted in 14 of 43 patients. Table 2 shows the relationship between the method of administration and the total dose given. Tumor regression was noted in two of 11 patients who received intratumoral treatment, and a decrease or disappearance of cancerous exudate was noted in four of seven patients receiving intracavitary treatment.

Subcutaneous injections of OK-432 failed to produce regression of lesions in remote sites but iv injections effected regression of tumor lesions in eight of 19 patients (table 2). Consequently, iv injections are now used chiefly.

There was no definite correlation between the total amount of OK-432 given and its effect. It is interesting that a beneficial result was obtained by the iv injection of a relatively small dose (table 2).

All responding patients had a high fever as a side effect of OK-432 treatment. The period between the start of the treatment and the appearance of the effect was variable (table 3).

Toxic Effects

The toxic reactions to this preparation are summarized in table 4. The most frequently occurring effect was a high fever which appeared in 35 patients (81%) and was encountered with all methods of administration. In most patients, a fever greater than 38 C (sometimes greater than 40 C) appeared 1–2 hours after the injection and was frequently accompanied by shaking chills. In most patients, the fever subsided after a few hours, but in some instances it continued for 24 hours. Fever did not inevitably occur after every injection. After the fever had subsided there was usually no pain, but occasionally slight general fatigue and anorexia remained for 1–2 days. Antifebriles were given but antihistamines and steroids were ineffective for pyrexia.

An increase in the leukocyte count to 10,000–20,000 cells/mm^3 was noted in 14 patients (33%). This increase was primarily due to the increase in neutrophils and was accompanied by a shift to the left. Slight anemia was noted in five patients.

Pain at the site of the injection occurred in five patients who were given local injection into the tumor and subcutaneous injection. This pain was variable, occurring at the time of injection in some patients and after 1–2 hours in others;

TABLE 3.—Patients treated effectively with OK-432

Patient No.	Age, sex	Diagnosis	Results of histologic test	Route of administration*	Total dose (KE)	Improvement	Histologic effect	Side effects	Previous treatment	Results
1	57, M	Carcinoma, stomach	Adenocarcinoma	It Ip	65 75	Decrease of hepatic tumor; disappearance of ascites	—	Fever	Mitomycin C, 5-FU, chromomycin A_3 hemisuccinate	Died 1 mo later
2	43, F	Carcinoma, gall bladder	Adenoacanthoma	It Ip	85 35	Decrease of Virchow's metastasis	Degeneration and necrosis of tumor cells	Fever, pain, leukocytosis	Mitomycin C, 5-FU	Died 1 mo later
3	49, M	Carcinoma, stomach	Adenocarcinoma	It	36	Decrease of hepatic tumor	Necrosis of tumor cells	None	Mitomycin C	Died 1 mo later
4	42, F	Carcinoma, stomach	—	Ip	50	Decrease of ascites	—	Fever, pain	Mitomycin C	Died 4 mos later
5	58, F	Carcinoma, breast (recurrent)	Adenocarcinoma	Ipl	100	Decrease of pleural fluid	Necrosis of tumor cells in lung and pleural cavity	Fever, leukocytosis	Mitomycin C	Died 4 mos later
6	43, M	Carcinoma, stomach	—	It	40	Decrease of hepatic tumor	Necrosis of tumor cells	Fever, pain	Mitomycin C, 5-FU	Died 2 mos later
7	47, F	Carcinoma, breast (recurrent)	Adenocarcinoma, tubulare medulare	Iv Ipl	1.8 30	Reduction of pleural fluid; decrease of hepatic tumor	—	Fever	Mitomycin C, 5-FU, chromomycin A_3 hemisuccinate	Died 10 mos later
8	73, M	Reticulosarcoma	—	Iv	10	Partial decrease of neck lymph gland	Degeneration of tumor cells	Fever	Vinblastine	Died 6 mos later
9	54, F	Carcinoma, breast (recurrent)	Adenocarcinoma scirrhus	Iv	19	Decrease of skin metastasis	Degeneration of tumor cells	Fever	Cyclophosphamide	Died 14 mos later
10	63, M	Carcinoma, stomach	Adenocarci-	Iv	9	Decrease of neck	Degeneration of	Fever	None	Died 8

10	63, M	Carcinoma, stomach	Adenocarcinoma, tubulare	Iv	9	Decrease of neck lymph gland; improvement of dysphagia	Degeneration of tumor cells	Fever, leukocytosis	None		Died 8 mos later
11	48, M	Carcinoma, stomach	Adenocarcinoma	Iv	12	Decrease of gastric tumor	—	Fever, leukocytosis	None		Alive at 7 mos
12	72, M	Carcinoma, stomach	Anaplastic adenocarcinoma	Iv	15	Decrease of gastric tumor; improvement of dysphagia	Degeneration and necrosis of tumor cells	Fever, leukocytosis	Bleomycin		Died 10 mos later
13	49, F	Carcinoma, lung	—	Iv	22	Improvement of tumor shadow	—	Fever, anemia	Mitomycin C, 5-FU	Alive at 5 mos	
14	62, F	Carcinoma, stomach (recurrent)	Adenocarcinoma	Iv	11	Decrease of neck lymph gland	—	Fever	Mitomycin C, 5-FU	Alive at 6 mos	

* It = intratumoral; ip = intraperitoneal; ipl = intrapleural; iv = intravenous.

TABLE 4.—Toxic effects in 43 patients treated with OK-432

Toxic effect	No. of patients	Percent
High fever	35/43	81.4
Pain	5/43	11.6
Anemia	5/43	11.6
Leukocytosis	14/43	32.6

it often disappeared immediately, or in some persisted for a few days. None of the patients had a deterioration in liver function.

None of the patients had newly developed proteinuria. Biopsied marrow patterns showed no special change before or after the administration of OK-432.

In a few cases, cultivation of *Streptococcus hemolyticus* from the blood or the injection site was attempted but all attempts gave negative results.

Case Reports

Patient No. 7.—Patient No. 7 was a 47-year-old woman who had had right breast cancer diagnosed 3 years and 2 months previously and who had undergone a right mastectomy in the Surgery Clinic of the Cancer Institute Hospital.

The course of her disease was uneventful, but about 4 months before hospitalization the patient had some difficulty in breathing and coughing during movement and had a fever around 38 C. A chest roentgenogram revealed hydrothorax in the right pleural cavity which was diagnosed as cancerous pleuritis by metastasis of mammary carcinoma after thoracic pleural puncture (fig 1). Thus, the patient was hospitalized 1 month later. At the time of hospitalization, her liver was palpable to a 5-finger width under the costal arch and a large round defect was seen in the liver scintiscan.

After hospitalization, the patient received digitalis, liver protective treatment, and combined treatment with mitomycin C, 5-FU, and chromomycin A_3, but no treatment effect was observed and her general condition became worse.

Treatment with OK-432 was then started and iv injections were given 5 times during 20 days for a total dose of 1.8 KE. Since this was the first patient to receive an iv injection, only a very minute amount of drug was given. About

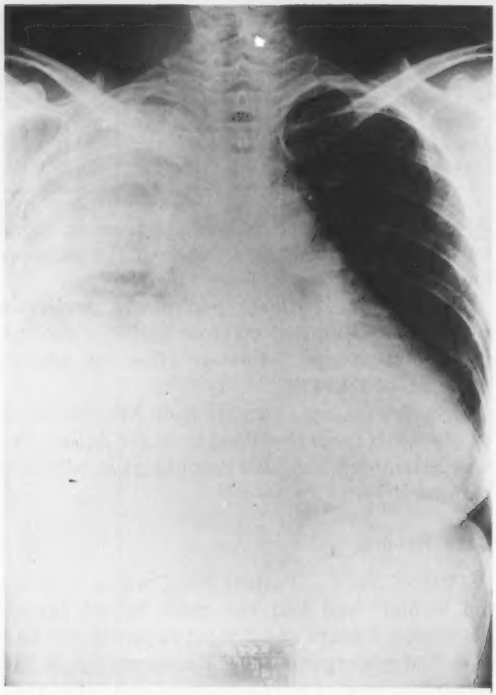

FIGURE 1.—Chest roentgenogram before treatment with OK-432 (patient No. 7).

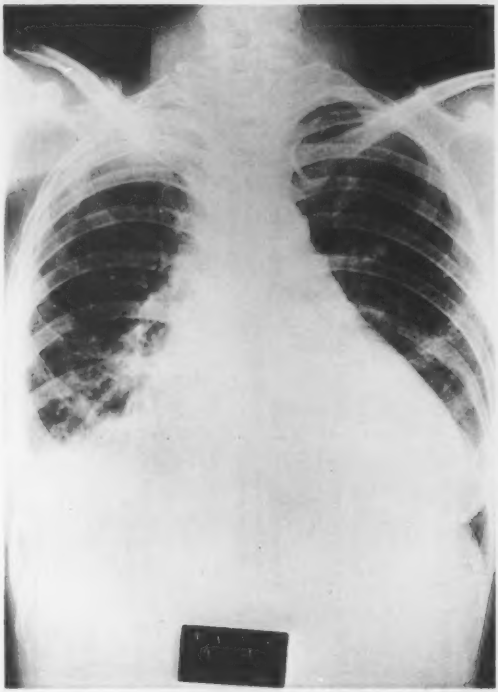

FIGURE 2.—Chest roentgenogram after treatment with OK-432 (patient No. 7).

5 weeks after the last injection, disappearance of the pleural fluid and a decrease in the size of the hepatic tumor (two-thirds decrease) were observed (fig 2), together with a reduction in the occurrence of dyspnea and coughing; however, the patient died 10 months after the treatment was stopped.

Patient No. 9.—Patient No. 9 was a 54-year-old woman who had left breast cancer and had received left mastectomy in the Surgery Clinic of the Cancer Institute Hospital. Cobalt irradiation treatment was given after the operation. One year later, she noticed bean-sized skin tumors scattered on her front chest, lower abdomen, and back, and she came as an outpatient to this hospital. Cyclophosphamide was administered orally but the number of skin tumors increased, with enlargement to thumb-tip size, and she was admitted to the Medical Clinic 10 months later.

At the time of hospitalization, there were 19 skin tumors and histologic examination revealed metastatic adenocarcinoma scirrhus. OK-432 was then given by iv injection, 8 times during 6 weeks, for a total dose of 19 KE. Two weeks after the injection, the size of the skin tumors decreased and their number was reduced to nine. A biopsied specimen indicated degeneration of the tumor cells, and numerous hyaline droplets were found in the tumor cells by PAS and Alcian Green staining. The occurrence of anorexia also decreased and she was discharged. She died 14 months later.

Patient No. 12.—Patient No. 12 was a 72-year-old man who had difficulty in swallowing and had been losing weight (about 10 kg) for 1 year. Six months earlier, his condition had been diagnosed as cancer of the gastric cardium, but he had left the hospital untreated. The difficulty of passage became so severe that he entered another hospital and was treated with

bleomycin for 5 weeks. Since there was no effect from the treatment, he was referred to and hospitalized in our Medical Clinic.

At the time of hospitalization, he could only swallow milk and fruit juice. X-ray examination of the esophagus and the stomach, and biopsy of the esophagus by a fiberscope, revealed the presence of anaplastic adenocarcinoma. He was given six iv injections of OK-432 during 1 month for a total dose of 15 KE. Extensive necrosis of the tumorous portion in the esophagus was observed by a fiberscopic examination, and the histologic picture (figs 3 and 4) showed degeneration of nuclei in tumor cells and fibrosis of the stroma. Improvement in swallowing difficulty and in his general condition occurred and he was discharged 1 week later. He died 10 months after leaving the hospital.

Patient No. 13.—Patient No. 13 was a 49-year-old woman who came to this hospital complaining of general fatigue and a decrease in body weight. Roentgenograms of the chest revealed a tumor-like round shadow in the right lower lung and an accumulation of pleural fluid. Pleural puncture produced a bloody exudate. Her condition was diagnosed as right lung cancer and cancerous pleuritis. After hospitalization, dyspnea increased.

Combined treatment with mitomycin C and 5-FU produced neither response in the tumor of the right lung or in hydrothorax accumulation, nor improvement in her general condition. OK-432 was given iv 5 times during 1 month for a total dose of 10 KE. The tumor shadow decreased to about one third its original size and the pleural fluid disappeared entirely after the injections. Improvement in the blood sedimentation rate and disappearance of dyspnea were noted. She died 8 months later of cerebral hemorrhage. Her postmortem examination showed necrosis of lung tumor.

DISCUSSION

As stated previously, it appears that OK-432 can produce objective improvement in clinical use. In our studies, the effect was not better than that produced by other existing drugs (table 5), but simple comparison of the effec-

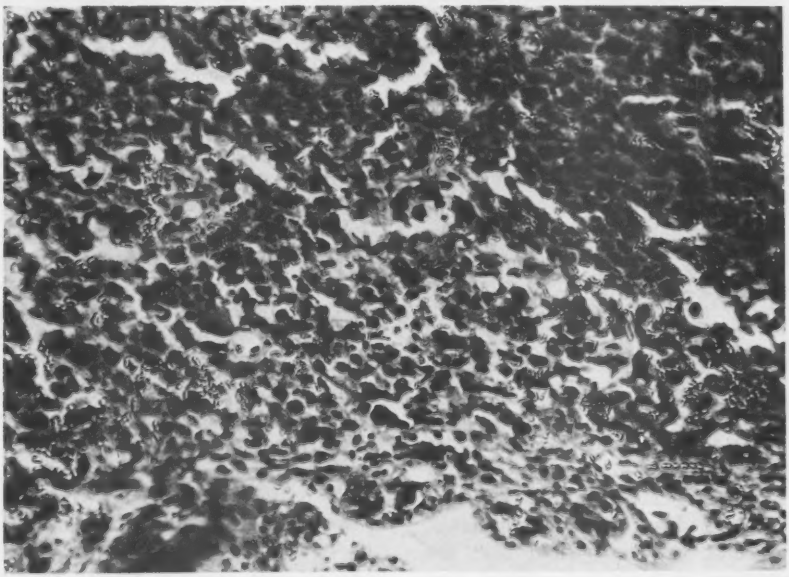

FIGURE 3.—Biopsied specimen of gastric tumor revealing anaplastic adenocarcinoma (patient No. 12).

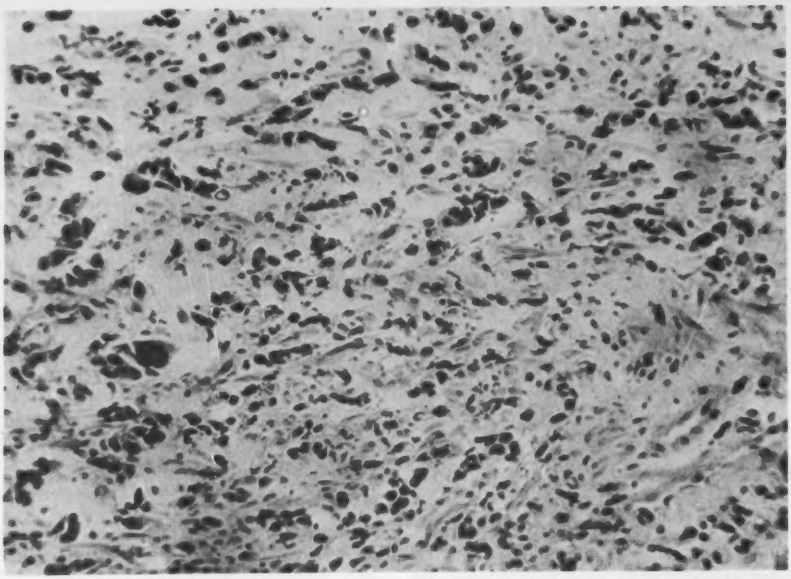

FIGURE 4.—Biopsied specimen of gastric tumor after treatment with OK-432 showing degeneration and necrosis of cancer cells (patient No. 12).

tiveness rates is of no significance. In any event, the objective improvement resulting from the use of OK-432 has been verified histologically, but these patients later had recurrences and many died; there has been no experience of long-term survival as yet. Nevertheless, OK-432 is a new type of anticancer agent that may open a new field of treatment, and although establishment of its clinical use is strongly desired, there are still some important problems to be solved.

The first of such problems is the elucidation of its carcinostatic mechanism.

Sakurai et al (4) observed that OK-432 was effective in rat Yoshida sarcoma and rat ascites hepatoma AH-13 in vitro, and in various rat ascites hepatomas and mouse Ehrlich carcinoma in vivo. They also found that pretreatment of rats with the preparation before injection of rat ascites hepatoma caused a marked prolongation of lifespan of tumor-bearing animals. From these results, they suggested that the preparation seemed to have both direct and host-mediated anticancer activity. Okamoto et al (3) presumed the destruction of RNA in cancer cells. Kurata et al (6) also reported that RNA synthesis of rat ascites hepatoma cells was suppressed by this preparation in vitro. According to the present clinical results, OK-432 does possess a cancerocidal action and this action has been verified by histologic observation.

The second problem, which is related to the first, is the extraction of the active component. This point is also related to the ease of administration and the abatement of side effects. Studies in this area are under way but problems remain to be solved in the future.

The third problem is the avoidance of side effects. There are no serious side effects according to our clinical results and experiments in animals; this holds true even for continued administration. OK-432 can be termed safe compared to other existing antitumor agents because it does not damage the bone marrow. However, a high fever in patients with advanced cancer, even if temporary, should be avoided. The fever is caused by the bacterial cells themselves, and the pain is occasionally so severe in topical application as to require the

TABLE 5.—Effectiveness of various anticancer drugs*

Anticancer drug	No. of patients	No. of improvements	Percent
ThioTEPA	19	2	11
Uracil mustard	23	2	9
Chromomycin A_3	59	3	5
SPI–77 (NSC–72274)	59	5	8
Cyclophosphamide	89	8	9
5-Fluorouracil	141	23	16
Mitomycin C	285	73	26
Vinca alkaloids	17	5	29
Bleomycin†	20	8	40
NSC–123109 (T–39)	22	1	5
NSC–84641 (No. 838)	12	0	0
OK–432	43	14	33
L-Asparaginase	7	2	29
Miscellaneous	107	6	6
Combination	213	53	25
Totals	1,116	205	19

*Treated at the Medical Clinic, Cancer Institute (Jan 1957–Mar 1970).
†Combined with radiotherapy.

discontinuation of medication. The cause of the pain might be related to the cells and not to the pH.

In some cases, there were decreases in the hemoglobin levels and erythrocyte counts and increases in the leukocyte counts after the administration of OK-432. There were no increases in serum protein levels, especially in the globulin content.

The repeated administration of OK-432 after a comparatively long interval failed to produce anaphylaxis-like shock. This suggests the absence or scarcity of an antigen-antibody reaction with the administration of OK-432.

Finally, there is the problem of the method of administration. At first, OK-432 was injected locally into the tumor and some excellent results were reported by the administration of a large dose (6). However, a principle of chemotherapy lies in systemic administration. This is why iv injection was tried after thoroughly confirming its safety, and it was found that regression in the distant focus can be obtained by this method. However, problems remain as to the route of administration, the amount of a single dose, the interval between administrations, and the total dose to be administered. The fact that increased dosage does not produce a better effect has been discussed previously. Larger doses often tend to aggravate the degree and duration of fever.

OK-432 is a new carcinostatic agent and further development may be anticipated.

LIST OF COMPOUNDS

L-Asparaginase: NSC-109229
Bleomycin: NSC-125066
Chromomycin A_3 hemisuccinate
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan
5-Fluorouracil: NSC-19893
Mitomycin C: NSC-26980
NSC-84641: 1-propanol, 3,3'-(methylimino)di-dimethanesulfonate, hydrochloride; methane sulfonate derivative; No. 838
NSC-123109: a mitomycin derivative; T-39
SPI-77: NSC-72274; podophyllic acid, 2-ethylhydrazide
ThioTEPA: NSC-6398; phosphine sulfide, tris(1-aziridinyl)-; triethylenethiophosphoramide
Uracil mustard: NSC-34462; uracil, 5-[bis(2-chloroethyl)amino]-
Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate

REFERENCES

1. BUSCH, W. Niederrheinische Gesellschaft für Natur- und Heilkunde in Bonn. Aus der Sitzung der Medicinischen Section vom 13. November 1867. Berl Klin Wochenschr 5:137–139, 1868.
2. COLEY, W. B. Contribution to the knowledge of sarcoma. Ann Surg 14:199–220, 1891.
3. OKAMOTO, H., SHOIN, S., KOSHIMURA, S., ET AL. Studies on the anticancer and streptolysin S-forming abilities of hemolytic streptococci (a review). Jap J Microbiol 11:323–336, 1967.
4. SAKURAI, Y., TSUKAGOSHI, S., SATOH, H., ET AL. Tumor-inhibitory effect of a streptococcal prepara-

tion (NSC-B116209). Cancer Chemother Rep 56: 9–17, 1972.

5. Bernheimer, A. W. Formation of a bacterial toxin (streptolysin S) by resting cells. J Exp Med 90: 373–392, 1949.

6. Kurata, S., Wakabayashi, K., and Ono, T. The effect of PC-B-45 on the RNA metabolism of tumor cell. Proc Japan Cancer Assoc, 29th Annual Meeting, 1970.

7. Toyota, B., Maesaka, A., Keyaki, Y., et al. Effect of streptococcal preparation "PC-B-45" on malignant head and neck tumors. J Otolaryngol Jap 72:1332–1338, 1969.

Clinical Trial With Mycophenolic Acid (NSC-129185), a New Antitumor Agent [1, 2]

S. Knudtzon and N. I. Nissen [3]

SUMMARY

Mycophenolic acid was recently found to be a potent antitumor agent with remarkably little toxicity in several experimental tumor systems. This report describes the first clinical use of mycophenolic acid. Twenty-three patients with various cancers were given mycophenolic acid, and of 17 patients treated for more than 21 days with daily oral doses ranging from 600 to 2400 mg/m², four patients had some objective response but did not qualify for partial remission. Since no systemic toxicity was observed, it is uncertain whether this dosage was within the therapeutic range; however, gastrointestinal intolerance prevented the use of larger oral doses. Other possible reasons for the poor response are discussed in this paper.

[Cancer Chemother Rep 56:221–227, 1972]

Mycophenolic acid was isolated from a Penicillium culture by Gosio in 1896. The molecular structure was determined by Birkinshaw in 1952 (1) (fig 1), and a scheme for the synthesis was devised by Birch and Wright in 1969 (2). Several workers have reported that mycophenolic acid shows limited antibacterial and antifungal activity (3–5). Interest in the drug was revived recently, when three laboratories independently found that it possessed considerable antiviral and antitumor activity (6–8).

In Earle's L strain cells in culture, Carter et al (8) demonstrated complete inhibition of mitosis by mycophenolic acid at a dose of 1 μg/ml, but no other signs of cellular toxicity occurred at concentrations as high as 100 μg/ml. Significant antitumor activity was found in vivo against various solid tumors such as Sarcoma 180, Lewis lung tumor, Carcinoma 755, Walker carcinosarcoma 256, and Yoshida tumor (solid and ascitic form). The therapeutic dose level was found to lie within a wide range (12.5–1000 mg/kg given orally daily for 7 days). L1210 leukemia was also sensitive to mycophenolic acid, whereas no significant inhibition could be demonstrated in Ehrlich, Landschütz, and LK lymphosarcoma ascites tumors.

Williams et al (9) reported that mycophenolic acid had a considerable effect in seven of 11 experimental solid tumors, among which Walker carcinosarcoma 256, Mecca lymphosarcoma, and X–5563 plasma cell myeloma proved to be most sensitive. The dose level was 100 mg/kg/day × 10 given intraperitoneally (ip). In L1210 leukemia, a 28% prolongation of survival time was found at a dose of 300 mg/kg given ip daily × 10. They also found antiviral activity of mycophenolic acid in vitro but none in vivo against vaccinia, influenza, and herpes simplex. Some activity was noted in two oncogenic viral infec-

[1] Received June 15, 1971; revised Nov 29, 1971; accepted Dec 15, 1971.

[2] Mycophenolic acid was prepared in the laboratories of Leo Pharmaceutical Products Trading, Ltd., Copenhagen, Denmark, from sterile filtrates of a Penicillium culture. Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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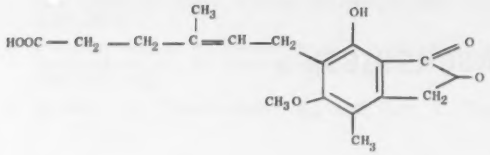

FIGURE 1.—Chemical structure of mycophenolic acid.

tions with Rous sarcoma virus and Friend virus (6,9,10).

Suzuki et al (7) found that mycophenolic acid had significant activity against several solid tumors in mice. Oral, ip, and subcutaneous routes of administration were equally effective. Furthermore, as an immunosuppressive agent, mycophenolic acid was found to be as potent as 6-mercaptopurine (6–MP) (11).

In our laboratory, the diethanolamine salt of mycophenolic acid given at a dose of 500 mg/kg ip daily × 5 increased the lifespan of mice receiving transplants of L1210 leukemia by 49%.

Mycophenolic acid is remarkable for its low degree of toxicity noted in animal experiments. In acute toxicity tests, the LD50 in the mouse was 2500 mg/kg with oral administration and 550 mg/kg with intravenous (iv) administration. The corresponding values for the rat were 700 and 450 mg/kg respectively (8).

Chronic toxicity tests were performed in rats and rhesus monkeys (8). The drug was administered in daily oral doses. All 20 rats survived a daily dose of 15 mg/kg for 23 weeks with only moderate anemia, but liver necrosis was found in three rats. With a dose of 30 mg/kg day, all rats died from pronounced anemia within 9 weeks, but neither thrombocytopenia nor leukopenia occurred, which is remarkable for an antitumor agent. Liver necrosis and lymphoid depletion were common. No toxic effects were noted in monkeys receiving daily doses of 50 mg/kg for 28 weeks. Although after 2 weeks of oral dosing at 150 mg/kg/day, blood-stained diarrhea and moderate hypoplastic anemia developed in monkeys, neither thrombocytopenia nor leukopenia was noted.

Subacute toxicity was evaluated in our laboratory by daily ip administration to mice for 5 days. The LD10 for the diethanolamine salt of mycophenolic acid was found to be 600 mg/kg.

The main mode of action of mycophenolic acid appears to be inhibition of guanine synthesis through interference with the activity of inosinic acid dehydrogenase (which converts inosinic acid to xanthylic acid) (8,12). Some cells may be able to bypass this block by using the "salvage" pathway of synthesizing guanylic acid from the free guanine. This synthesis is blocked by 6–MP, and a potentiating effect of 6–MP on mycophenolic acid is also confirmed in experiments using the Ehrlich ascites tumor (8).

It has recently been reported that mycophenolic acid is rapidly inactivated in the organism by conjugation to glucuronic acid, and it was hypothesized that only tumors with a high β-glucuronidase activity would be sensitive to the drug (13). There is no proof as yet on this point.

As far as we are aware, there have been no published reports of clinical trials of mycophenolic acid against human cancer.

MATERIALS AND METHODS

Due to its poor solubility in water, mycophenolic acid was used in the form of the diethanolamine salt. Capsules for oral treatment contained 250 mg of the salt, an amount equal to 188 mg of the free acid. (All the results reported here refer to the diethanolamine salt.) For iv treatment, vials of 500 mg of sterile dry powder were reconstituted before use in 50 ml of citrate-phosphate buffer (pH 7.4) and administered directly by iv injection. The same concentration (1%) was used for intrapleural and ip treatment.

Since no human toxicity data were available, the initial clinical dose had to be based on animal toxicity data. Estimation of human maximal tolerated dose (MTD), using the conversion factors of Freireich et al (14), gave figures ranging from 50 mg/kg (mouse data) over 17–50 mg/kg (monkey data) to 2–4 mg/kg (rat data). Accordingly, the first oral dose used was 3 mg/kg or 150 mg/m^2.

The first six patients were given mycophenolic acid at a dose of 150 mg/m^2/day orally for 1 week. When this elicited no toxic symptoms,

TABLE 1.—Patient data: treatment with mycophenolic acid

Patient No.	Age, sex	Diagnosis and stage	Prior therapy	Treatment			Duration of treatment (days)	Other treatment	Tumor response/ duration
				Schedule	Maximal dose (g/m^2/day)	Total dose (g/m^2)			
1	74, F	Reticulum cell sarcoma, IV	—	Daily, oral	0.7	121.1	175		35% reduction/ 110 days
2	66, M	Reticulum cell sarcoma, IV	X-ray therapy (neck)	Daily, oral	0.8	7.9	15		Progression
3	78, F	Reticulum cell sarcoma, II	—	Daily, oral	1.0	42.1	42		25% reduction/ 28 days
4	60, M	Reticulum cell sarcoma, III	—	Daily, oral	1.3	28.6	41		Progression
5	64, F	Reticulum cell sarcoma, III	X-ray therapy (mantle)	Daily, oral	1.3	70.1	64	Vitamin A (150,000 units daily for 2 wks)	Progression
6	74, M	Reticulum cell sarcoma, IV	X-ray therapy (neck)	Continuous iv infusion	6.0	12.0	2		No change
7	68, M	Reticulum cell sarcoma, IV	X-ray therapy (abdomen)	Twice weekly, iv	0.5	3.5	24		Progression
8	24, M	Hodgkin's disease, III	BCNU, prednisone, vincristine, procarbazine, chlorambucil, vinblastine	Daily, oral	1.2	25.6	36	Radiotherapy (2 wks), 6-MP	Progression
9	44, F	Hodgkin's disease, IV	X-ray therapy (mantle)	Daily, oral	1.3	30.7	41		No change
10	60, M	Hodgkin's disease, II; recurrence in irradiated area	X-ray therapy (abdomen)	Daily, oral	1.4	89.7	74		No change
11	25, F	Hodgkin's disease, II; recurrence in irradiated area	X-ray therapy (mantle)	Daily, oral	2.4	182.7	80		50% reduction/ 53 days
12	59, F	Lymphosarcoma, IV	Vincristine, prednisone, cyclophosphamide, X-ray therapy (mediastinum)	Thrice weekly, intrapleural	1.3	2.4	5	Prednisone	Progression

13	75, F	Lymphosarcoma, IV	X-ray therapy (neck)	Daily, oral	1.1	152.1	157	Vitamin A (150,000 units daily for 2 mos)	25% reduction/ 103 days
14	59, M	Chronic lymphocytic leukemia	—	Daily, oral	0.7	10.0	23		Progression
15	74, F	Chronic lymphocytic leukemia	Prednisone, X-ray therapy (spleen)	Daily, oral	1.0	22.9	43	Allopurinol	Progression
16	67, M	Multiple myeloma	BCNU, prednisone	Daily, oral	1.2	21.8	22		Progression
17	51, F	Multiple myeloma	—	Daily, oral	1.3	25.2	22		Progression
18	53, M	Chronic granulocytic leukemia	—	Daily, oral	0.9	10.4	16	Allopurinol	Progression
19	57, M	Gastric carcinoma, metastatic	Surgical treatment, thioTEPA (ip)	Daily, oral Ip × 1	1.1 1.1	58.7 1.1	62 1		Progression
20	67, F	Gastric carcinoma, inoperable	Exploratory laparotomy, vincristine, 5-FU, cytosine arabinoside, cyclophosphamide, prednisone	Daily, oral	1.1	45.0	42		Progression
21	53, M	Esophageal cancer, inoperable	Exploratory laparotomy, vincristine, 5-FU, cytosine arabinoside, cyclophosphamide, prednisone	Daily, oral Daily, iv	1.4 0.7	9.8 9.1	7 13		Progression
22	79, M	Gastric carcinoma, inoperable	Exploratory laparotomy	Daily, oral	1.4	55.2	40		Progression
23	47, F	Gastric carcinoma, inoperable	Exploratory laparotomy, 5-FU, cytosine arabinoside, vincristine, cyclophosphamide, methotrexate, mitomycin C, prednisone	Daily, oral Weekly, ip	1.5 1.5	40.9 11.7	27 56		Progression

the doses were escalated every week in steps of 150–300 mg/m^2. The next patient received treatment at the 300 mg/m^2 level, the next three at the 450 mg/m^2 level, the next four at the 600 mg/m^2 level, the next two at the 900 mg/m^2 level, and the final four at the 1200 mg/m^2 level. In these patients doses were escalated every 3–7 days in steps of 150–300 mg/m^2. Ten patients received maintenance therapy at a constant oral dose level ranging between 750 and 2400 mg/m^2 for more than 4 weeks (one patient received 750 mg/m^2, three patients received 900 mg/m^2, four patients received 1200 mg/m^2, one patient received 1500 mg/m^2, and one patient received 2400 mg/m^2). All doses were calculated to the nearest 250-mg amount (one capsule).

Treatment was interrupted when (*a*) no effect was observed after treatment for more than 3 weeks, or (*b*) the disease progressed rapidly despite treatment.

During a pharmacologic study, one patient received mycophenolic acid at a dose of 6 g/m^2 (10 g, total dose) in a continuous 24-hour iv infusion and others received smaller iv single doses. In an attempt to control a pleural exudate, one patient received mycophenolic acid intrapleurally and two patients received the drug ip for a carcinomatous ascites.

A total of 23 patients were treated with mycophenolic acid. The diagnosis, prior therapy, treatment schedule, and dose and duration of treatment are listed in table 1.

All patients had a pretreatment physical examination including measurements of palpable tumors. The following pretreatment studies were made: chest roentgenograms, skeletal survey, and lymphangiograms when indicated; a complete blood count; serum alkaline phosphatase; serum glutamic oxaloacetic transaminase and bilirubin; fasting blood sugar; serum electrophoresis; serum uric acid; serum creatinine; urinalysis. These studies were repeated weekly for 1 month, and thereafter when indicated. Marrow aspiration was done repeatedly in patients who had leukemia and multiple myeloma.

Cases of patients with multiple myeloma were evaluated by following the abnormal serum protein level, the urinary protein excretion rate, skeletal survey, and the plasma cell count in the marrow. In patients with chronic lymphocytic and granulocytic leukemias, the complete blood count and size of the spleen were used as parameters. Blood transfusions and antibiotics were given as indicated. Patients with increased serum uric acid were given allopurinol.

Since very little pharmacologic information is obtainable from the literature, the blood level and urinary excretion rate of mycophenolic acid were determined by a spectrofluorometric method as reported in the second part of this communication (15).

RESULTS

Toxicity

Treatment with mycophenolic acid was well tolerated. No depression of bone marrow function was found as expressed by constant hemoglobin levels and white blood cell and thrombocyte counts. There were no toxic effects on liver and kidney function. Central nervous system toxicity from mycophenolic acid, recently reported (16), was not experienced in our patients. Neither alopecia nor phlebitis was noted. The only side effects during oral treatment were mild gastrointestinal symptoms, mainly nausea, which increased in frequency when the dose level was greater than 900 mg/m^2. Two patients complained of local pain after ip administration of 2000 mg, and one patient had similar complaints locally after intrapleural administration.

Response

The results of mycophenolic acid therapy are listed in table 1. Among 17 patients treated for more than 3 weeks with daily oral doses ranging from 600 to 2400 mg/m^2, 13 patients showed no response or progressive disease and only four had a moderate reduction in tumor size. Patient No. 1, who had generalized reticulosarcoma and had not been treated previously, obtained a gradual improvement and a reduction of axillary and retroperitoneal lymphomas which by Day 65 reached 35%. This reduction and a stable nonsymptomatic condition still continued 110 days later when this study

was terminated. In the main part of her course of treatment the mycophenolic acid dose level was 700 mg/m²/day. In patient No. 3, who had reticulosarcoma, a 25% reduction in the lymphomas was noticed after 14 days of oral mycophenolic acid therapy (1000 mg/m²/day). Twenty-eight days later, during continued therapy, the nodes increased in size. Before this study, patient No. 11 had received radiotherapy for stage II Hodgkin's disease. She was treated with mycophenolic acid because of a recurrence in the irradiated area. After 27 days of treatment, the axillary and mediastinal lymphomas were reduced 50% in size, but on Day 80, after continued therapy with mycophenolic acid at a dose of 2400 mg/m²/day, she relapsed due to the development of a new Hodgkin's lesion. Patient No. 13, who had generalized lymphosarcoma, obtained only a slight reduction of less than 25% of the retroperitoneal lymphomas, but this reduction and a stable nonsymptomatic clinical condition still persisted when the study was terminated 103 days later. After dose escalation in the first 3 weeks, she received mycophenolic acid at a dose of 1100 mg/m²/day.

No responses resulted from the iv, intrapleural, or ip treatments.

DISCUSSION

The effectiveness of mycophenolic acid in a wide spectrum of experimental animal tumors and its low toxicity in animals suggested that mycophenolic acid might be useful also in the treatment of human cancer.

Our study confirmed the low toxicity of the compound, but only a slight antitumor effect could be demonstrated with the dosage and preparation used. Only one patient approached a partial remission rating, and three others had an objective improvement which might have been spontaneous.

The possibility exists that the dose level used in our study is too low to demonstrate an antitumor effect of mycophenolic acid, but a further escalation of the dose level was not possible due to the gastric symptoms and the local pain mentioned previously.

Poor absorption, rapid degradation, or rapid excretion of the drug are among the many possible explanations for the poor results. Metabolic studies, reported in the second part of this communication (15), showed good resorption of mycophenolic acid but a rapid conversion in vivo to the glucuronide salt. This finding, which also has been reported by others (13), would suggest that only tumors which contained membrane-bound β-glucuronidase in quantities sufficient for splitting the presumably inactive glucuronide would respond to the treatment. It has been reported that malignant epithelial tumors generally contain more β-glucuronidase than mesenchymal tumors (17) and myelomas have also been found to contain high levels of the enzyme (18). However, our four patients with gastric carcinoma, one patient with esophageal carcinoma, and two patients with multiple myelomas had no response to mycophenolic acid.

The possibility that the tumor cells are using the "salvage" pathway mentioned previously for the synthesis of guanylic acid is another explanation for the lack of response to mycophenolic acid.

LIST OF COMPOUNDS

Allopurinol:NSC–108836; 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol, monosodium salt

BCNU: NSC–409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Chlorambucil: NSC–3088; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cyclophosphamide: NSC–26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

Cytosine arabinoside: NSC–63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C

5-Fluorouracil: NSC–19893; 5-FU

6-Mercaptopurine: NSC–755; purine-6-thiol, monohydrate; 6-MP

Methotrexate: NSC–740; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Mitomycin C: NSC–26980

Mycophenolic acid: NSC–129185; 4-hexenoic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-, (*E*)-

Prednisone: NSC–10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC–77213; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

ThioTEPA: NSC–6398; phosphine sulfide, tris(1-aziridinyl)-

Vinblastine: NSC–49842; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC–67574; leurocristine, sulfate (1:1)

REFERENCES

1. BIRKINSHAW, J. H., RAISTRICK, H., and ROSS, D. J. Studies in the biochemistry of micro-organisms. 86. The molecular constitution of mycophenolic acid, a metabolic product of *Penicillium brevi compactum* Dierckx. Part 3. Further observations on the structural formula for mycophenolic acid. Biochem J 50:630–634, 1952.
2. BIRCH, A. J., and WRIGHT, J. J. A total synthesis of mycophenolic acid. J Chem Soc Section D (Chemical Communications) No. 14, 1969, pp 788–789.
3. ABRAHAM, E. P. The effect of mycophenolic acid on the growth of *Staphylococcus aureus* in heart broth. Biochem J 39:398–408, 1945.
4. FLOREY, H. W., JENNINGS, M. A., GILLIVER, K., ET AL. Mycophenolic acid. An antibiotic from *Penicillium Brevi-Compactum* Dierckx. Lancet 1: 46–49, 1946.
5. NOTO, T., SAWADA, M., ANDO, K., ET AL. Some biological properties of mycophenolic acid. J Antibiot 22:165–169, 1969.
6. WILLIAMS, R. H., LIVELY, D. H., DELONG, D. C., ET AL. Mycophenolic acid: antiviral and antitumor properties. J Antibiot 21:463–464, 1968.
7. SUZUKI, S., KIMURA, T., ANDO, K., ET AL. Antitumor activity of mycophenolic acid. J Antibiot 22:297–302, 1969.
8. CARTER, S. B., FRANKLIN, T. J., JONES, D. F., ET AL. Mycophenolic acid: an anti-cancer compound with unusual properties. Nature 223:848–850, 1969.
9. WILLIAMS, R. H., BOECK, L. D., CLINE, J. C., ET AL. Fermentation, isolation, and biological properties of mycophenolic acid. Antimicrob Agents Chemother 1968, pp 229–233.
10. CLINE, J. C., NELSON, J. D., GERZON, K., ET AL. In vitro antiviral activity of mycophenolic acid and its reversal by guanine-type compounds. Appl Microbiol 18:14–20, 1969.
11. MITSUI, A., and SUZUKI, S. Immunosuppressive effect of mycophenolic acid. J Antibiot 22:358–363, 1969.
12. FRANKLIN, T. J., and COOK, J. M. The inhibition of nucleic acid synthesis by mycophenolic acid. Biochem J 113:515–524, 1969.
13. SWEENEY, M. J., HOFFMAN, D. H., and POORE, G. A. Possible in situ activation of mycophenolic acid by β-glucuronidase. Cancer Res 31:477–478, 1971.
14. FREIREICH, E. J, GEHAN, E. A., RALL, D. P., ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.
15. LINTRUP, J., HYLTOFT-PETERSEN, P., KNUDTZON, S., ET AL. Metabolic studies in man with mycophenolic acid (NSC–129185), a new antitumor agent. Cancer Chemother Rep 56:229–235, 1972.
16. ANDO, K., SUZUKI, S., and ARITA, M. Synthesis of mycophenolic acid β-D-glucuronide and its antitumor activity. J Antibiot 23:408–413, 1970.
17. MONIS, B., BANKS, B. M., and RUTENBURG, A. M. β-D-Glucuronidase activity in malignant neoplasms of man. A histochemical study. Cancer 13:386–393, 1960.
18. MANDEL, H. G., and RALL, D. P. The present status of cancer chemotherapy—A summary of papers delivered at the Cherry Hill Conference on "A Critical Evaluation of Cancer Chemotherapy." Cancer Res 29:2478–2485, 1969.

Metabolic Studies in Man With Mycophenolic Acid (NSC-129185), a New Antitumor Agent [1, 2]

J. Lintrup, P. Hyltoft-Petersen, S. Knudtzon, and N. I. Nissen [3]

SUMMARY

The metabolism of mycophenolic acid in man was studied with a spectrophotofluorometric assay method. Mycophenolic acid is readily absorbed from the digestive tract and rapidly conjugated to mycophenolic acid β-glucuronide. After a single intravenous (iv) dose was given, the initial half-life of free mycophenolic acid in serum was found to be approximately 4–12 minutes. Observations of serum levels of total mycophenolic acid, free mycophenolic acid, and mycophenolic acid β-glucuronide were made after single-dose iv administration of 500 and 1000 mg of the drug, after continuous infusion of 10 g/24 hours for 48 hours, after a single oral dose of 1000 mg, and after intrapleural administration of 2000 mg. The latter administration led to the most prolonged serum level of free mycophenolic acid. Urinary excretion took place in the form of the mycophenolic acid glucuronide and was essentially complete within 24 hours after a single iv dose.

[Cancer Chemother Rep 56:229–235, 1972]

In the preceding report (1) we reviewed the literature on mycophenolic acid (fig 1) and reported our own experience from a clinical trial. In summary, mycophenolic acid is a nontoxic Penicillium antibiotic with antiviral and antitumor activity demonstrable in tissue culture and animal studies. However, our clinical results revealed only a weak antineoplastic effect against the tumors tested. Possible explanations for this discrepancy from the animal results are poor absorption, rapid degradation, or excretion of the active compound. Of importance in this respect is the report of the in vivo inactivation of mycophenolic acid by conjugation to glucuronic acid with formation of the β-glucuronide (2). We therefore undertook a study of the assay method of mycophenolic acid and its application to studies of mycophenolic acid metabolism in man.

ANALYTIC METHOD [4]

Choice of Method

Gas chromatographic (3), thin-layer chromatographic (4), and microbiological (5,6) methods for the assay of mycophenolic acid have

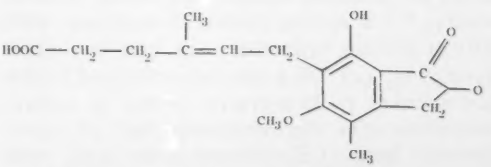

FIGURE 1.—Structural formula of mycophenolic acid.

[1] Received June 15, 1971; revised Nov 29, 1971; accepted Dec 15, 1971.

[2] Mycophenolic acid: 4-hexenoic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-, (*E*)-.

[3] Department of Clinical Chemistry (Drs. Lintrup and Hyltoft-Petersen) and Department of Internal Medicine (Drs. Knudtzon and Nissen), Finsen Institute, Copenhagen, Denmark.

[4] We are grateful to Leo Pharmaceutical Products Trading Ltd., Copenhagen, Denmark, for valuable suggestions.

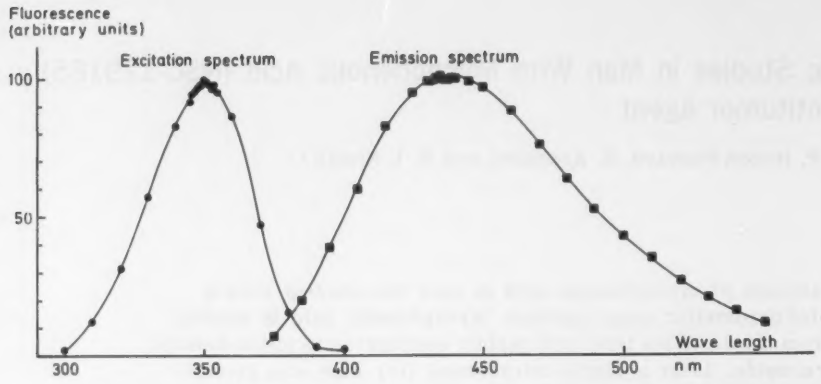

FIGURE 2.—Excitation and emission spectra of mycophenolic acid in 0.01 M NaOH.

recently been published. Only the latter describes a method for an accurate determination of mycophenolic acid in low concentrations. However, a microbiological method may not be applicable to differential analysis of free and conjugated mycophenolic acid. Since the drug has a rather strong fluorescence in alkaline solution, we chose to base our assays on spectrophotofluorimetry. The primary and secondary fluorescence spectra are given in figure 2. In acid solution the fluorescence is weaker by approximately 2 orders of magnitude and the spectra are somewhat different. Mycophenolic acid is readily extracted with chloroform at a moderately acid pH and may be reextracted into a small volume of strongly alkaline solution. The β-glucuronide is not extracted and may be determined as the difference between analysis results before and after the application of β-glucuronidase to the sample.

Reagents

The following reagents were used: (*a*) 1.5 M sodium dihydrogen phosphate; (*b*) chloroform, pro analysis, washed twice with one-fifth volume 0.1 M sodium hydroxide and once with 0.01 M sodium hydroxide; (*c*) 0.01 M sodium hydroxide; (*d*) DEA-mycophenolic acid standard solution (1.25 mg/ml) in 0.01 M sodium hydroxide (the diethanolamine salt of mycophenolic acid [DEA-mycophenolic acid] was obtained from Leo Pharmaceutical Products Trading Ltd., Copenhagen, Denmark [5]); (*e*) 0.2 M acetate buffer, pH 4.4; (*f*) β-glucuronidase solution, 2500 Fishman units/ml, in acetate buffer (*e*). β-Glucuronidase was obtained from Calbiochem, Inc. (No. 34743). Reagents were kept at temperatures of +4 C and were used for up to 2 months.

Apparatus

A Carl Zeiss spectrofluorimeter with an XBO 450 W high pressure Xenon lamp and two M4Q III prism monochromators were used. The primary was set at 350 nm with a slit width of 0.3 mm and the secondary at 435 nm with a slit width of 2.0 mm. Nearly maximal amplification was applied.

Standardization

Standardization was made individually for each sample. Two μl of the mycophenolic acid standard solution (*d*), ie, 2.50 μg mycophenolic acid, was added to a 2.00-ml portion of the final solution. The measured increase in fluorescent energy was used as a reference standard in the calculation of the amount of mycophenolic acid in the sample being studied.

Serum Samples

Serum was separated by centrifugation shortly after the venipuncture and stored at

[5] Elementary analysis was in good accordance with the theoretical formula $C_{17}H_{20}O_6N$, melting point was 104.5–107 C, and thin-layer chromatography in a cyclohexane-chloroform-methanol-acetic acid system showed no more than one spot.

–20 C for up to 1 month if convenient. Serum (1.00 ml) was diluted with 1.50 ml sodium dihydrogen phosphate solution (*a*) and extracted once with 25 ml of chloroform (*b*). The chloroform was separated by means of filtration through siliconized paper (Whatman 1 PS) and extracted within 4.00 ml of the sodium hydroxide solution (*c*). After another separation using siliconized paper, 2.00 ml of the aqueous layer was pipetted off for the purpose of standardization mentioned in the previous section, and the rest was transferred to the cuvette for measurement. Readings were taken at 5-second intervals and the mean of the readings from 10 to 40 seconds was used in the calculation. If a result exceeded 8 μg/ml, the analysis was repeated on serum diluted 1:10 with acetate buffer (*e*).

Urine Samples

Urine samples were processed as described for serum except that only 0.100 ml of urine was used.

Total Mycophenolic Acid in Serum and Urine

Serum (0.100 ml) or urine (0.010 ml) was diluted 1:10 with acetate buffer (*e*), 0.020 ml β-glucuronidase (*f*) was added, and the test tube was incubated for 30 minutes at 37 C. The succeeding procedure was as previously described.

Comments on Analytic Method

The quality of the chloroform is critical. Even analytic grades may, in our experience, contain impurities that seriously impede the determination of mycophenolic acid by giving rise to (*a*) high blank values, (*b*) a rapid decline in mycophenolic acid fluorescence during the measurement, and/or (*c*) an enhancement of the fluorescence of mycophenolic acid. Fortunately, the significance of these interferences is easily reduced by the alkaline washings of the chloroform (*b*) mentioned in the section on reagents. As a matter of precaution, we feel it is essential to check the reagent blank, to take several successive meter readings to test fluorescence stability, and in each case to verify the increase in fluorescence evoked by a standard addition of mycophenolic acid.

The slit on the primary monochromator should be kept as narrow as possible to attain fluorescence stability during the light exposure to the measurement. Figure 3 shows the results of an extreme experiment on this point.

The standard curve is linear to about 4 μg of mycophenolic acid/ml corresponding to a serum concentration of about 16 μg/ml. Results are reproducible within 5%–10%. Recovery of mycophenolic acid added to serum samples in amounts from 2.5 to 12.5 μg/ml is about 0.85 (table 1). Repeating the extraction procedures does not improve this figure significantly. We have made no corrections for incomplete recovery. Samples have been kept for up to 2 months at –20 C without any apparent change in the result of the analysis.

TABLE 1.—Control of analytic method: recovery of mycophenolic acid added to normal serum

Serum sample	Added drug (μg)	Before addition (μg/ml)	After addition (μg/ml)	Recovered drug (μg)	Recovery fraction
A	2.5	1.45	3.65	2.20	0.88
A	5.0	1.45	6.12	4.67	0.93
A	7.5	1.45	8.32	6.87	0.92
A	10.0	1.45	10.63	9.18	0.92
A	12.5	1.45	13.05	11.60	0.93
B	10.0	1.54	10.78	9.24	0.92
C	10.0	0.58	9.00	8.42	0.84
D	10.0	0.55	8.90	8.35	0.84
E	10.0	0.49	8.39	7.90	0.79
F	10.0	0.38	8.47	8.09	0.81

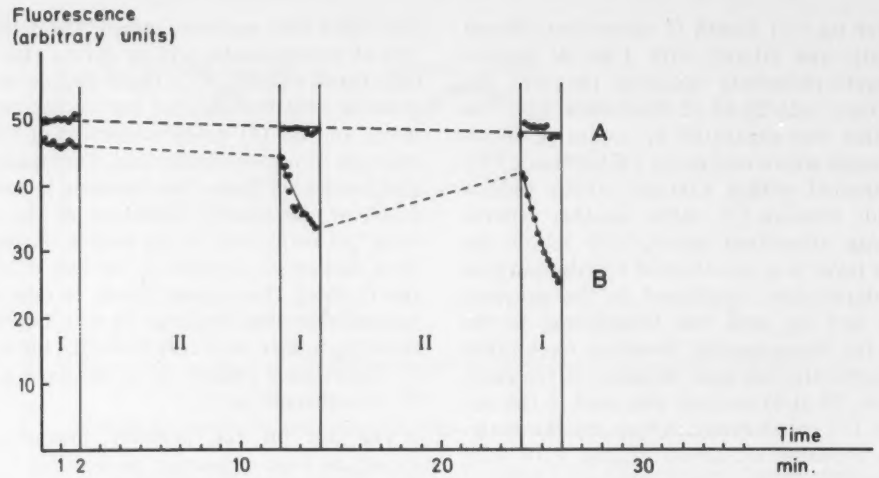

FIGURE 3.—Dependence of stability of mycophenolic acid fluorescence (2.5 μg/ml) on the choice of slit widths. Slits of primary and secondary monochromators set at 0.3 and 2.0 mm (A) and 2.0 and 0.3 mm (B). The measurements were carried out in 3 periods of 2 minutes, with 10-minute intervals. I = Periods of light exposure inherent to measurements. II = Periods of darkness during stand-by.

The procedure is not very specific for mycophenolic acid. Serum samples from persons not treated with the drug have given results ranging from 0.4 to 1.5 expressed as micrograms of mycophenolic acid per milliliter of serum. In urine samples this blank value is generally 1 order of magnitude higher. This nonspecific fluorescence shows no distinct maximum and is probably caused by many different substances which we have not investigated further. For measurements on samples from patients receiving treatment with mycophenolic acid, we have, in all instances, subtracted the pretreatment value. This way of correcting for the sample blank is not completely satisfactory but at present we see no other practical solution. Differential spectrofluorimetry with readings at several primary and/or secondary wavelengths will hardly improve the specificity substantially, since the spectral maxima of mycophenolic acid are rather broad (fig 2). The lack of specificity is of minor importance for the accuracy of the measured peak levels that for most patients are 10–100 times the non-specific level. But the accuracy becomes poor as the pretreatment level is approached.

The amount of β-glucuronidase and the time used for the cleavage of the glucuronide represent a safe excess even at high levels of glucuronide in the samples of urine.

METABOLIC STUDIES IN PATIENTS

Due to its poor solubility in water, mycophenolic acid was administered in the form of the diethanolamine salt. The same salt was used as the standard of analysis. Accordingly, the results refer to this substance with no correction for the departure in molecular weight.

Serum Concentration

After a single intravenous (iv) dose, the concentration of free mycophenolic acid falls very rapidly (fig 4). In two patients given 500 and 1000 mg of mycophenolic acid respectively, initial half-lives of 4 and 12 minutes were noted and the decreases to the detection level of about 1 μg/ml were accomplished in 30 and 120 minutes respectively. The first patient with Hodgkin's disease had normal renal and liver function. The second patient had normal renal function but a slightly elevated serum glutamic oxaloacetic transaminase level, which proved during autopsy to be due to biliary cirrhosis.

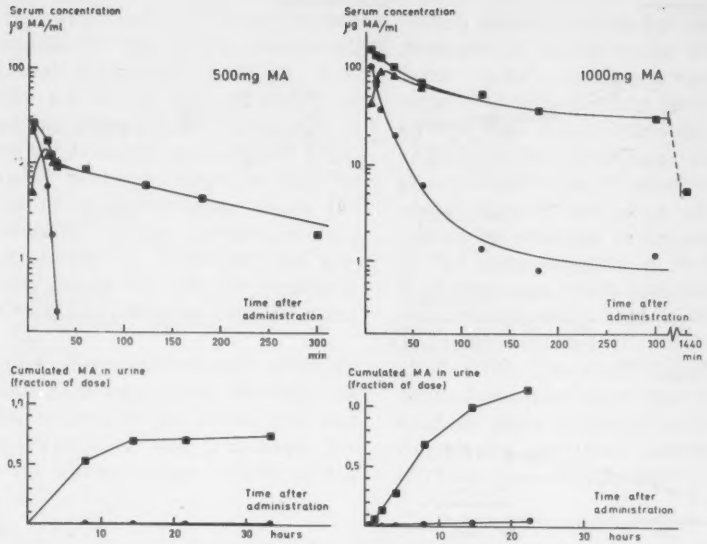

FIGURE 4.—Serum concentrations and urine excretion after iv administration of 500 and 1000 mg mycophenolic acid in 2 patients. Serum concentrations of free (●) and total mycophenolic acid (■) and the calculated values for mycophenolic acid glucuronide (▲) are shown in the upper part of the figure. The cumulated urine excretion of free (●) and total mycophenolic acid (■) are shown in the lower part.

Application of β-glucuronidase in the analysis revealed the presence of mycophenolic acid glucuronide. The concentration of this conjugate equaled that of free mycophenolic acid as early as 10 minutes after the injection, and by 30 minutes its fraction was found to be 0.97 and 0.80 in the two experiments.

Continuous Iv Administration

One patient received continuous iv administration at a dose rate of 10 g/24 hours for 48 hours. Concentration levels comparable to those noted during the first 5 hours (30–100 µg mycophenolic acid/ml) after a single iv dose of 1000 mg were maintained. Even here the glucuronide fraction prevailed, constituting from 0.73 to 0.84 at different times. A few hours after the cessation of the administration the serum concentrations dropped to low values. The patient had generalized reticulum cell sarcoma with normal renal and liver function.

Intrapleural Administration

After intrapleural administration of 2000 mg to one patient, a rather stable serum concentra-

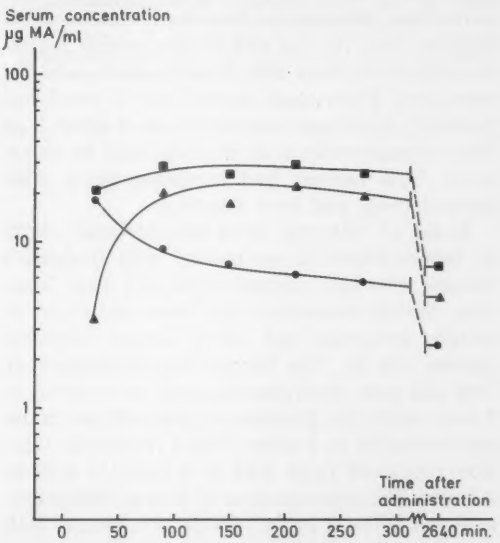

FIGURE 5.—Serum concentrations after intrapleural administration of 2000 mg mycophenolic acid. Serum concentrations of free (●) and total mycophenolic acid (■) and the calculated values for mycophenolic acid glucuronide (▲) are shown.

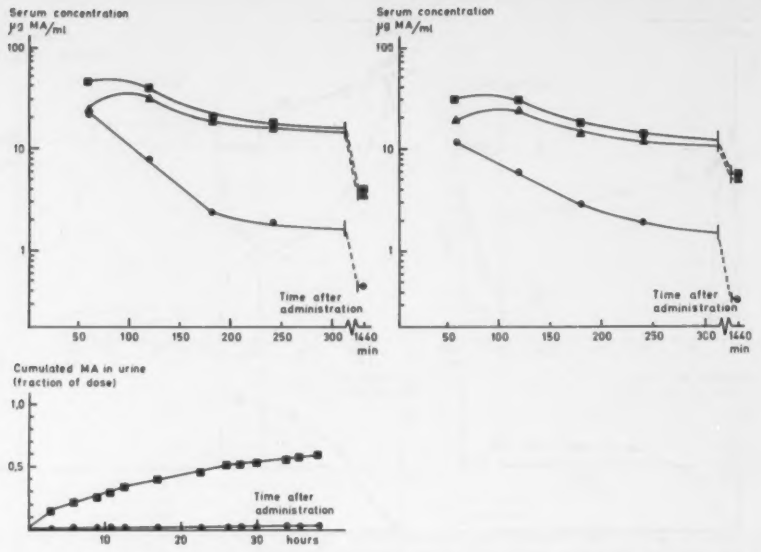

FIGURE 6.—Serum concentrations and urine excretion after oral administration of 1000 mg of mycophenolic acid to the same patient on 2 occasions. See legend for figure 4 for definition of symbols.

tion of 20–30 µg total mycophenolic acid/ml was measured over 5 hours (fig 5). Through this period the glucuronide fraction increased from 0.16 to 0.78. At the end of the period a concentration of 6 µg free mycophenolic acid/ml remained. Forty-eight hours later a small but probably significant concentration of about 2 µg free mycophenolic acid/ml could still be measured. This patient had lymphosarcoma with normal renal and liver function.

Doses of 1000 mg were administered orally on two occasions to one patient with Hodgkin's disease who had normal renal and liver function. Serum concentrations were measured at hourly intervals and gave almost identical curves (fig 6). The highest concentrations of free and total mycophenolic acid were found at 1 hour while the glucuronide reached maximum concentration at 2 hours. The glucuronide fraction increased from 0.60 at 1 hour to 0.90 at 4 hours. The concentration of free mycophenolic acid fell below 2 µg/ml within 4 hours on both occasions.

Urine Excretion

Urinary excretion of mycophenolic acid is rapid and essentially complete within 24 hours. In the two experiments using single iv administration, 75% and 117% of the given dose were recovered in the urine (fig 4). The recovery after oral administration was 60% (fig 6). Essentially all mycophenolic acid is excreted in the form of the glucuronide conjugate. The small and inconsistent amounts measured as free mycophenolic acid (up to 5%) were probably due to the lack of accuracy inherent in the blank correction.

The figures do not allow any proper calculation of the renal clearance of mycophenolic acid glucuronide because the serum concentration varied so rapidly. On a very rough estimate the results are consistent with a clearance of about 100 ml/minute.

DISCUSSION

The results indicate that mycophenolic acid is readily absorbed from the digestive tract, rapidly conjugated to the mycophenolic acid glucuronide, and excreted in the urine within 24 hours as mycophenolic acid glucuronide.

After iv administration half of the mycophenolic acid will be conjugated within 10

minutes and the target cell will therefore only be briefly exposed to the active compound. Mycophenolic acid glucuronide is usually considered inactive due to its lack of ability to cross the cell membrane (2) (although a single report to the contrary has appeared [7]), and mycophenolic acid can therefore only be expected to be of value in cases where the tumor cell contains enough membrane-associated β-glucuronidase to hydrolyze the glucuronide in situ, unless the free mycophenolic acid level can be kept sufficiently high by other means.

In 18 transplantable animal tumors, Sweeney et al (2) found good correlation between the β-glucuronidase activity of the tumor cells and response to mycophenolic acid treatment. We did not assay the glucuronidase activity of the human tumors treated, but found no significant response to mycophenolic acid in two tumor types, gastric carcinoma and multiple myeloma (1), both of which have been reported to have a high β-glucuronidase activity.

Methods for increasing the fraction of free mycophenolic acid in serum might prove more useful, but if formation of the glucuronide cannot be retarded, iv infusion of 5–10 g/day or the same quantity in 6–8 fractional daily oral doses may prove sufficient to maintain the free mycophenolic acid level of greater than 1 μg/ml, reported effective in tissue culture studies (8). The single experiment with intrapleural administration may indicate that some kind of depot preparation may be useful, although the quantities necessary will seriously limit the clinical applicability.

REFERENCES

1. KNUDTZON, S., and NISSEN, N. I. Clinical trial with mycophenolic acid (NSC–129185), a new antitumor agent. Cancer Chemother Rep 56:221–227, 1972.
2. SWEENEY, M. J., HOFFMAN, D. H., and POORE, G. A. Possible in situ activation of mycophenolic acid by β-glucuronidase. Cancer Res 31:477–478, 1971.
3. GAINER, F. E., and WESSELMAN, H. J. GLC of mycophenolic acid and related compounds. J Pharm Sci 59:1157–1159, 1970.
4. GAINER, F. E., and HUSSEY, R. L. Thin-layer chromatography of mycophenolic acid and related compounds. J Chromatogr 54:446–448, 1971.
5. NOTO, T., SAWADA, M., ANDO, K., ET AL. Some biological properties of mycophenolic acid. J Antibiot 22:165–169, 1969.
6. NOTO, T., HARADA, Y., and KOYAMA, K. A turbidimetric bioassay method for determination of mycophenolic acid. J Antibiot 23:96–98, 1970.
7. ANDO, K., SUZUKI, S., and ARITA, M. Synthesis of mycophenolic acid β-D-glucuronide and its antitumor activity. J Antibiot 23:408–413, 1970.
8. CARTER, S. B., FRANKLIN, T. J., JONES, D. F., ET AL. Mycophenolic acid: an anti-cancer compound with unusual properties. Nature 223:848–850, 1969.

Adriamycin (NSC-123127) in the Treatment of Acute Myelocytic Leukemia [1, 2, 3]

Engracio P. Cortes, Rose Ruth Ellison, and Jerome W. Yates [4]

SUMMARY

Adriamycin, a new cytotoxic antibiotic, was used to treat 15 adults who had acute myelocytic leukemia. One of the 12 patients studied attained complete remission twice and no other patients responded. The toxic effects of adriamycin administration were: severe bone marrow depression, oral ulcerations and stomatitis, alopecia, transient electrocardiogram changes, fever, and chemical thrombophlebitis. It is hoped that the efficacy of adriamycin will be increased when the drug is used in combination with other chemotherapeutic agents in the treatment of previously untreated patients.

[Cancer Chemother Rep 56:237–243, 1972]

Adriamycin,[5] a new cytotoxic antibiotic isolated from cultures of *Streptomyces peucetius* var *caesius* by Farmitalia Research Laboratories, belongs to the anthracycline group of antibiotics, as does daunomycin. The two antibiotics differ in structure only at the 14-position: a hydrogen atom at that position in daunomycin is replaced by a hydroxyl group in adriamycin (1) as shown in figure 1.

Comparative studies of adriamycin and daunomycin (2) revealed that when equally toxic doses (2 and 2.5 mg/kg of adriamycin and 2.5 and 3.25 mg/kg of daunomycin) were given to C3H mice bearing lymphosarcoma, the survival time was longer in mice receiving adriamycin than in those receiving daunomycin. The inhibitory dose (ID50) of adriamycin was 1.5 mg/kg; the ID50 of daunomycin was 3.3 mg/kg. Adriamycin, therefore, seemed to have a higher therapeutic index than daunomycin (1.25 and 0.67 respectively).

The initial clinical studies by Bonadonna et al (3–5) demonstrated that adriamycin had an antitumor effect in several types of solid tumors in children, and in acute lymphoblastic leukemia and malignant lymphomas in both children and adults. Subsequent clinical studies by Tan et al (6), and additional clinical data by Bonadonna et al (7), confirmed that adriamycin had a wide spectrum of antitumor activity. Despite these clinical studies, no trial of the activity of adriamycin in acute myelocytic leukemia (AML) has been cited. It is the purpose of this paper, therefore, to report our experience with 15 adult patients with AML treated with adriamycin.

MATERIALS AND METHODS

Adriamycin was supplied as an orange-red crystalline material readily soluble in water. Each vial contained 10 mg of powder which was to be dissolved in 10 ml of 0.9 saline, resulting in a concentration of 1 mg/ml. The calculated dose was injected directly into the tubing of a

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[2] Supported by research grants CA–2599, CA–5834, and CA–10044 from the National Cancer Institute, Public Health Service.
[3] Adriamycin was supplied by Farmitalia Research Laboratories, Milan, Italy, through Dr. James F. Holland.
[4] Department of Medicine A, Roswell Park Memorial Institute, Buffalo, NY.
[5] Chemical nomenclature and NSC numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

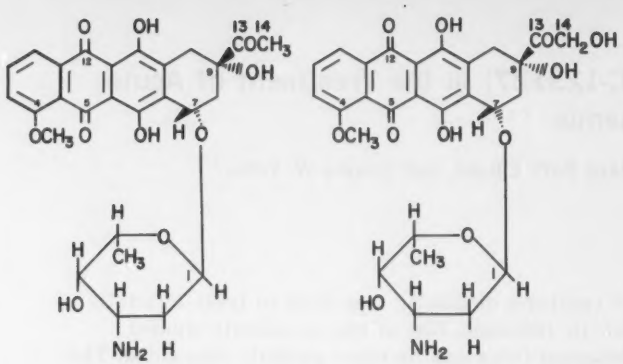

FIGURE 1.—Structural formulas of daunomycin (left) and adriamycin (right).

running intravenous saline infusion. Care was taken to avoid extravasation of the drug in order to prevent chemical thrombophlebitis and necrosis.

Patients were initially randomly allocated to receive 20 mg/m² of adriamycin, given by one of several schedules, as part of a phase I study. Because no drug activity was observed in the first three patients with AML treated with this dose, some of the subsequent patients with this diagnosis were given 35 mg/m². Two dose schedules, therefore, were used:

a. 20 mg/m²/day × 3, followed by a 4-day rest period. The 3-day treatment was then repeated if no toxicity had occurred by that time.

b. 35 mg/m²/day × 3, followed by a 4-day rest period. The 3-day treatment was then repeated if no toxicity had occurred by that time.

Maintenance treatment was to be started when remission was attained. In the absence of remission, ie, when the blast cells in the bone marrow persisted in excess of 25% or increased after the patient recovered from hematologic toxicity, the study was terminated.

Electrocardiograms (ECG) were done weekly while the patient was in the induction phase of observation. The value of a protected environment was being studied concomitantly and patients were randomly allocated into this study prior to the start of treatment with adriamycin. Thus, some, but not all, of the patients were isolated during the period of severe bone marrow depression. Platelet concentrates were administered daily, if possible, when bleeding occurred or when the platelet count fell to less than 10,000/mm³, even in the absence of hemorrhagic manifestations. White blood cell (wbc) transfusions were given prior to or at the nadir of leukopenia, especially in the presence of fever, if compatible donors were available. When fever occurred, attempts were made to isolate the causative organism, although treatment with intravenously (iv) administered cephalothin plus kanamycin or polymycin or gentamycin was started before the results of the cultures had been obtained. Appropriate antibiotics were given when the organisms were identified.

Fifteen patients who had received prior antileukemic chemotherapy were treated. All had AML, but two had an erythroleukemia variant and one had myelomonocytic cells.

The response to therapy was evaluated according to the criteria of Acute Leukemia Group B (8) and its recent modification: only complete or partial remissions are considered significant. Remissions of marrow or peripheral blood or both in the presence of progression or persistence of disease elsewhere, ie, node involvement, hepatosplenomegaly, are not considered to be remission. Four categories of response are measured: bone marrow (M), hemogram (H), physical examination (P), and symptoms and performance (S). Each cri-

terion is graded from 1 (normal) to 4 (serious impairment).

Disease status A requires a rating of "1" in all four categories, ie, bone marrow, hemogram, physical examination, and symptoms and performance. There must be improvement to disease status A for a response to be considered a complete remission, which is symbolized as M_1–H_1–P_1–S_1. To be rated M_1, bone marrow must be normocellular with no more than 5% blast cells and no more than 10% blast cells plus promyelocytes; there must be normal erythropoiesis (more than 15% erythroid elements), granulopoiesis (more than 25% normal granulocytic elements including myelocytes, metamyelocytes, stabs, and segmented polymorphonuclear leukocytes), and normal thrombopoiesis. In addition, the peripheral blood must be free of evidence of leukemia with a hemoglobin level of at least 12 g/100 ml in adult men (11 g/100 ml for women), at least 2000 mature neutrophilic granulocytes/mm^3, no blast cells or other leukemic cells, and at least 100,000 platelets/mm^3.

Disease status B implies a rating of "2" in one or more of the four categories, but no rating of "3" in any category. For such a rating, the bone marrow in a patient with AMI may contain 5.1%–25% blast cells. A rating of "2" for the hemogram implies a hemoglobin level of at least 9 g/100 ml (with maintenance of this level not attributable to transfusion), at least 1000 mature neutrophilic granulocytes/mm^3, 50,000–99,000 platelets/mm^3, and no more than 5% blast cells in the peripheral blood.

RESULTS

Only one of the 15 adults with AML treated with adriamycin had a complete remission, and no partial remissions resulted. Three of the 15 patients died before they had completed the course of adriamycin treatment. Their deaths (Days 2, 2, and 6), were secondary to infection or subarachnoid hemorrhage.

All of the patients had received antileukemic therapy prior to treatment with adriamycin; six of these patients had received daunomycin (total doses ranging from 30 to 300 mg/m^2), alone or in combination with other chemotherapeutic agents. Two of the six patients who had previously received daunomycin had responded to it with partial remissions lasting 53 and 362 days. The one patient who had a complete remission with adriamycin had not received daunomycin previously.

Case Report (fig 2).—An 18-year-old white male college student, was found to have AML in December 1969. He was induced into complete remission with cytosine arabinoside and 6-thioguanine in 1 month's time. Although he received maintenance treatment with the same drugs, he had a relapse 3 months after his marrow had reached the M_1 stage. At the time of relapse, he had 9750 wbc/mm^3, with 27% blast cells, 225,000 platelets/mm^3, and a hypercellular bone marrow containing 93.6% myeloblasts. He was placed in reverse isolation and "gut sterilization" (by means of oral doses of nystatin, gentamycin, and vancomycin) was started. Adriamycin treatment at a dose of 35 mg/m^2/day $\times$ 3, was started on April 23, 1970. On the eighth day, the patient had a markedly hypocellular marrow that coincided with an elevation in body temperature. Material was obtained for identification of possible causative micro-organisms but no pathogens grew in culture. Antibiotic treatment was started (by the parenteral route) and the patient was given packed red cells, platelet concentrates, and leukocyte transfusions for support. With the recovery of his bone marrow to M_1 status, his temperature returned to normal. A remission, complete in all respects but hemoglobin level, was attained on the 22nd day of study, and complete remission was reached 32 days later. Adriamycin maintenance treatment was started on Day 33 at a dose of 35 mg/m^2/day $\times$ 2 and was repeated on Day 76. No decrease in bone marrow cellularity was noted during the maintenance phase of observation, although leukopenia (wbc of 1200/mm^3) was observed on Day 89. On Day 96, when the wbc was 3700/mm^3, an M_2 marrow with 9.4% myeloblasts and 4.4% promyelocytes was noted. Autologous modified leukemic cells were given intradermally on that day. An M_1 marrow was noted 8 days later, but after another 2 weeks, ie, on Day 118, relapse was evident with 19.4% blast cells in the marrow aspirate. A second course of treatment with adriamycin, at a total dose of

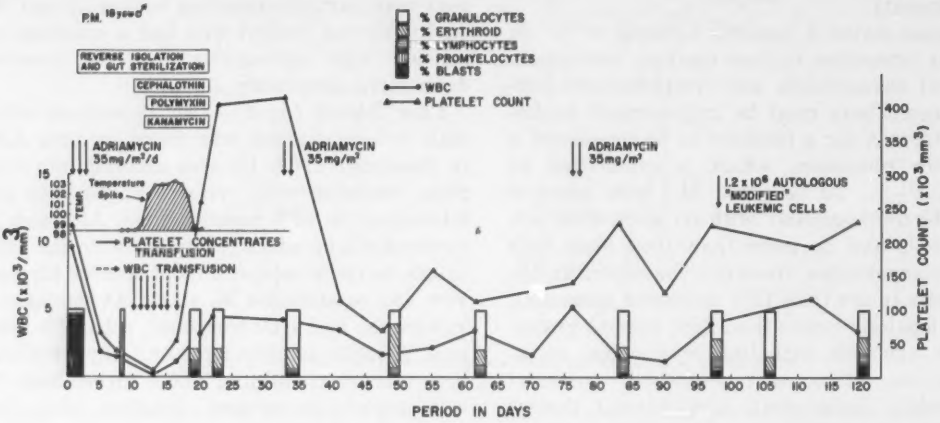

FIGURE 2.—Complete remission in a previously treated patient with acute myelogenous leukemia.

175 mg/m² given by the same schedule, produced a second remission on Day 31. The patient's remission was maintained with methotrexate and 6-mercaptopurine, but relapse occurred 36 days later.

Adriamycin toxicity is summarized in table 1. The most significant effect of adriamycin administration was bone marrow depression, the nadir of which occurred 8–23 days after treatment. Five patients, who had total doses of adriamycin ranging from 60 to 210 mg/m², attained markedly hypocellular marrows. Moderate marrow hypocellularity developed in five patients and two patients retained their pretreatment cellularity, despite the occurrence of leukopenia and thrombocytopenia. All 12 patients had platelet counts less than 100,000/mm³ at the start of treatment. Ten patients had a wbc nadir less than 1000/mm³ and eight had platelet counts less than 10,000/mm³. The depression of the platelet counts paralleled that of the wbc. Recovery of the platelets and wbc was noted between the 19th and 33rd day of study, ie, 7–14 days after the nadir was reached in all but one patient. This patient had not recovered from bone marrow aplasia at the time of his death which was due to massive and diffuse gastrointestinal hemorrhage with superimposed subdural hemorrhage on Day 50.

Stomatitis, characterized by tenderness and swelling of the tongue, or ulcerations of the buccal mucosa and palate, or both, developed in ten patients and usually was severe, resulting in dysphagia even for oral liquids. These symptoms occurred during the stage of bone marrow depression. Ten patients who had moderate to pronounced marrow hypocellularity had toxic reactions as described above; however, the two patients who retained their pretreatment marrow cellularity did not have stomatitis or oral ulcerations, despite the occurrence of leukopenia. Mild nausea was noted in four patients. Only one patient vomited after receiving the drug; this occurred a few minutes after the treatment.

Fever occurred in ten patients and coincided with the development of leukopenia and stomatitis. One patient, however, had fever despite the persistence of a normocellular marrow and the absence of stomatitis. Pseudomonas grew in a culture of material obtained from the throat of one patient. She died, and bronchopneumonia was confirmed at necropsy. Another patient had bacteroides septicemia, and died of massive gastrointestinal bleeding. Another patient had pseudomonas-related cellulitis of the scrotum but he responded well to antibiotic treatment. No pathogens were recovered in cultures from seven patients. The patients' temperatures

TABLE 1.—Toxic effects noted after administration of adriamycin

Patient No.	Bone marrow depression			Other toxic effects †				
	Cellularity *	Leukopenia or increased leukopenia	Thrombocytopenia	Stomatitis	Alopecia	ECG changes	Fever	Thrombophlebitis
1	2+	+	+	0	+	0	0	0
2	1+	+	+	+	+	0	0	0
3	1+	+	+	+	+	0	+	0
4	0	+	+	+	+	+	+	0
5	1+	+	+	+	+	+	+	0
6	0	+	+	+	+	+	+	0
7	2+	+	+	0	+	0	+	0
8	0	+	+	+	+	0	+	0
9	0	+	+	+	+	0	+	+
10	0	+	+	+	+	0	+	0
11	1+	+	+	+	+	0	+	0
12	1+	+	+	+	+	0	+	0
Total		12	12	10	12	3	10	1

* 2+ = normocellular; 1+ = moderately hypocellular; 0 = markedly hypocellular.
† 0 = no toxic effect; + = toxic effect noted.

usually returned to normal during bone marrow recovery.

Alopecia occurred in all patients. Hair loss started 2–3 weeks after therapy; however, new growth was observed in 2–3 months, even in the one patient who received adriamycin maintenance treatment.

Two of the six patients who had had total cumulative doses of daunomycin ranging from 30 to 420 mg/m² with no consequent cardiotoxicity, had changes in their electrocardiograms after they had received total induction doses of adriamycin of 105–210 mg/m². One of the four patients who had not had daunomycin prior to treatment with adriamycin, had cardiotoxic reactions after receiving 210 mg/m² of adriamycin. ECG changes occurred at the peak of bone marrow and gastrointestinal reactions to adriamycin. Two patients had ST–T wave changes suggesting an ischemic pattern, and the third had prolonged QT intervals with no accompanying hypocalcemia or other possibly causative abnormalities. The ECG changes were transient in two patients. In the third, the T wave inversion converted to normal during bone marrow recovery, but after more drug had been given (174 mg/m² total), a repeat ECG showed sinus bradycardia of 55 beats/minute with occasional preventricular ectopic beats. The latter reverted to normal sinus rhythm despite administration of an additional dose of 70 mg/m² of adriamycin. No chest pain or shortness of breath was experienced by the patient.

Chemical thrombophlebitis developed in one patient, occurring where the adriamycin had extravasated. This resulted in an indurated, erythematous, non-tender fibrosis, and contracture of the wrist.

No abnormalities in liver or kidney function that might be ascribed to adriamycin toxicity were noted.

The larger dose of adriamycin produced more severe marrow depression and oral toxicity than the smaller dose.

DISCUSSION

The mechanism of action of adriamycin, unlike that of daunomycin (9–13), has not been fully investigated. Both drugs were found to have antitumor effect in animal Ehrlich ascites tumors, nitrosomethylurea-induced lymphosarcoma, Oberling-Guérin-Guérin myeloma, and Sarcoma 180 (2,14). Clinical studies revealed that daunomycin produced brief remissions in patients with neuroblastoma, reticulum cell sarcoma, rhabdomyosarcoma (15), and malignant lymphomas (16–18). Daunomycin alone or in combination with other chemotherapeutic agents, however, was found to be useful in inducing remission in acute lymphocytic (15,19–

25) and myelocytic leukemia (26–34). Despite the effectiveness of daunomycin in treating leukemia, the potential cardiotoxicity of the drug (15,28,34,35,37) is a major concern. This toxicity has occurred after a cumulative total dose of 25 mg/kg in children (15), and 2–8 mg/kg or more in adults (36). Boiron et al (31), however, experienced only one episode of daunomycin cardiotoxicity in a study of 64 patients with AML treated with daunomycin (total doses ranging from 36 to 38 mg/kg). Other toxic effects of daunomycin were severe bone marrow depression, alopecia, skin rash, and gastrointestinal disturbances, including stomatitis, nausea, and vomiting.

Unlike daunomycin, adriamycin was found to have an antitumor effect not only in neuroblastoma, malignant lymphomas, and acute lymphocytic leukemia, but also in other solid tumors in children and adults, including Ewing's sarcoma, Wilms' tumor, choriocarcinoma (in women), osteogenic sarcoma, and bronchogenic carcinoma (3–7,38). No clinical comparison of daunomycin and adriamycin has been undertaken currently.

The toxic effects of adriamycin observed in our series were similar to those reported by Bonadonna et al (3–5,7) and Tan et al (6). The most consistent toxic effect of adriamycin was bone marrow depression. All marrow elements were affected, thus, intensive supportive therapy during the induction period was required. The wbc and platelets were the most susceptible elements, and depression in cell counts occurred even in the absence of discernible changes in bone marrow cellularity.

Adriamycin produced more severe buccal mucosa ulcerations and alopecia than did daunomycin. Alopecia and leukopenia can occur with a small dose of adriamycin, ie, total dose of 60 mg/m², with no detectable decrease in bone marrow cellularity. Chemical thrombophlebitis leading to wrist contracture was noted in one patient. A running intravenous infusion is compulsory for the administration of adriamycin.

Cardiotoxicity resulting from adriamycin treatment was transient and was manifested by ECG changes after total induction doses of 105–210 mg/m² had been given. This toxicity did not appear to be related to previous exposure to daunomycin. Postmortem examination of the three patients with ECG changes failed to reveal any myocardial degeneration or fibrosis, such as that seen in daunomycin cardiotoxicity (37).

Adriamycin was used to treat 15 adults with AML. Six of the 15 patients treated had previously received daunomycin. One of the 15 responded with a complete remission. It is possible that the use of adriamycin in combination with other chemotherapeutic agents may prove to be more effective in the treatment of previously untreated patients. Adriamycin, like daunomycin, may be active primarily as an induction drug.

ADDENDUM

Mathé et al (39) reported recently that adriamycin given alone produced six complete and three partial remissions in 18 cases of previously treated AML.

LIST OF COMPOUNDS

Adriamycin: NSC–123127
Cytosine arabinoside: NSC–63878; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunomycin: NSC–2151; daunorubicin (NSC–83142)
Gentamycin: NSC–82261; gentamicin, sulfate
6-Mercaptopurine: NSC–755; purine-6-thiol, monohydrate
Methotrexate: NSC–740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Nystatin: NSC–150817
6-Thioguanine: NSC–752; purine-6-thiol, 2-amino-

REFERENCES

1. Arcamone, F., Cassinelli, G., Fantini, G., et al. Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from *Streptomyces peucetius* var. *caesius*. Biotechnol Bioeng 11:1101–1110, 1969.
2. Di Marco, A., Gaetani, M., and Scarpinato, G. Adriamycin (NSC–123,127): a new antibiotic with antitumor activity. Cancer Chemother Rep 53:33–37, 1969.
3. Bonadonna, G., Monfardini, S., De Lena, M., et al. Clinical evaluation of adriamycin, a new antitumor antibiotic. Br Med J 3:503–506, 1969.
4. Bonadonna, G., Monfardini, S., De Lena, M., et al. Phase I and preliminary phase II evaluation of adriamycin (NSC–123127). Cancer Res 30: 2572–2582, 1970.
5. Monfardini, S., Bonadonna, G., Di Pietro, S., et al. Sperimentazione clinica preliminare con adriamicina in pazienti affetti da leucemia e tumori solidi in fase avanzata. [Clinical trials with adriamycin in leukemia and solid tumors.] Tumori 55: 197–216, 1969.
6. Tan, C., Wollner, N., King, O., et al. Adriamycin,

a new antibiotic in treatment of childhood leukemia and other malignant neoplasms. Proc Am Assoc Cancer Res 11:79, 1970.
7. BONADONNA, G., and MONFARDINI, S. Therapeutic effects of adriamycin in the neoplastic disease of children and adults. Proc Am Assoc Cancer Res 11:10, 1970.
8. ELLISON, R. R., HOLLAND, J. F., WEIL, M., ET AL. Arabinosyl cytosine: a useful agent in the treatment of acute leukemia in adults. Blood 32:507–523, 1968.
9. SILVESTRINI, R., DI MARCO, A., DI MARCO, S., ET AL. Azione della daunomicina sul metabolismo degli acidi nucleici di cellule normali e neoplastiche coltivate in vitro. [The action of daunomycin on the metabolism of nucleic acids of normal and neoplastic cells growing "in vitro".] Tumori 49:399–412, 1963.
10. BARBIERI, P., DI MARCO, A., MAZZOLENI, R., ET AL. Azione della daunomicina sul metabolismo degli acidi nucleici nell' E. coli B. G Microbiol 12:71–82, 1964.
11. DI MARCO, A. Daunomycin in pharmacological activity at the cellular level. Pathol Biol 15:897–902, 1967.
12. HARTMANN, G., GOLLER, H., KOSCHEL, K., ET AL. Hemmung der DNA-abhängigen RNA- und DNA-Syntheses durch Antibiotica. Biochem Z 341:126–128, 1964.
13. THEOLOGIDES, A., YARBRO, J. W., and KENNEDY, B. J. Daunomycin inhibition of DNA and RNA synthesis. Cancer 21:16–21, 1968.
14. DI MARCO, A., GAETANI, M., DORIGOTTI, L., ET AL. Daunomycin: a new antibiotic with antitumor activity. Cancer Chemother Rep 38:31–38, 1964.
15. TAN, C., TASAKA, H., YU, K.-P., ET AL. Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease. Clinical evaluation with a special reference to childhood leukemia. Cancer 20:333–353, 1967.
16. AMIEL, J.-L., SCHWARZENBERG, L., SCHNEIDER, M., ET AL. Valeur de la rubidomycine dans le traitement de la maladie de Hodgkin. [Value of rubidomycin in the treatment of Hodgkin's disease.] Pathol Biol 15:961–962, 1967.
17. BOURDIN, J. S., CLAVEL, B., SARACINO, R. T., ET AL. Traitement des hémato-sarcomes par la rubidomycine. [Treatment of hematosarcoma with rubidomycin.] Pathol Biol 15:963–965, 1967.
18. CHAUVERGNE, J., and LAGARDE, C. Chimiothérapie anticancéreuse par rubidomycine. (Note préliminaire sur 14 observations). [Anticancer chemotherapy with rubidomycin. Preliminary report on 14 cases.] Pathol Biol 15:969–970, 1967.
19. MATHÉ, G., SCHWARZENBERG, L., SCHNEIDER, M., ET AL. Acute lymphoblastic leukemia treated with a combination of prednisone, vincristine, and rubidomycin. Lancet 2(7512):380–382, 1967.
20. HOLTON, C. P., LONSDALE, D., NORA, A. H., ET AL. Clinical study of daunomycin (NSC–82151) in children with acute leukemia. Cancer 22:1014–1017, 1968.
21. HOLTON, C. P., VIETTI, T. J., NORA, A. H., ET AL. Clinical study of daunomycin and prednisone for induction of remission in children with advanced leukemia. N Engl J Med 280:171–174, 1969.
22. JONES, B., ET AL. Daunomycin in the treatment of resistant acute lymphoblastic leukemia. Proc 10th Ann Am Soc Hemat, Toronto, Canada, 1967.
23. JONES, B. Daunomycin in the treatment of resistant acute lymphoblastic leukemia. (Abstr) Blood 30: 890–891, 1967.
24. JONES, B. Daunorubicin vs. daunorubicin + prednisone vs. daunorubicin + vincristine + prednisone in resistant childhood leukemia. Proc Am Assoc Cancer Res 11:41, 1970.
25. RAUSEN, A. R., and GLIDEWELL, O. J. Combination of chemotherapy with L-asparaginase, prednisone, vincristine and daunomycin in advanced childhood leukemia. Proc 13th Int Cancer Congr, Munich, Germany, 1970, p 94.
26. DREYFUS, B., SULTAN, C., and BESSON, P. Note sur le traitement de six leucémies aigües myéloblastiques par la rubidomycine. [Note on the treatment of six cases of acute myeloblastic leukemia with rubidomycin.] Pathol Biol 15:925–927, 1967.
27. HOLLAND, J. F. Daunomycin treatment in acute leukemia. Pathol Biol 15:929–932, 1967.
28. MALPAS, J. S., and SCOTT, R. B. Rubidomycin in acute leukaemia in adults. Br Med J 3:227–229, 1968.
29. BERNARD, J., JACQUILLAT, C., BOIRON, M., ET AL. Essai de traitement des leucémies aiguës lymphoblastiques et myéloblastiques par un antibiotique nouveau: la rubidomycin (13 057 RP). Presse Med 75:951–955, 1967.
30. DREYFUS, B., SULTAN, C., BOIRON, M., ET AL. Sur le traitement d'attaque par la rubidomycine de 19 cas de leucémie aiguë myéloblastique. Presse Med 78:55–57, 1968.
31. BOIRON, M., JACQUILLAT, C., and WEIL, M. Daunorubicin in the treatment of acute myelocytic leukaemia, Lancet 1(7590):330–333, 1969.
32. BORNSTEIN, R. S., THEOLOGIDES, A., and KENNEDY, B. J. Daunorubicin in acute myelogenous leukemia in adults. JAMA 207:1301–1306, 1969.
33. BERNARD, J., BOIRON, M., HOLLAND, J. F., ET AL. Rubidomycin (daunomycin) in the treatment of acute granulocytic leukemia. Proc Am Assoc Cancer Res 9:5, 1968.
34. SERPICK, A. A., and HENDERSON, E. S. Observations on toxicity and clinical trials with daunomycin. Pathol Biol 15:909–912, 1967.
35. MACREZ, C., MARNEFFE-LEBREQUIER, H., RIPAULT, J., ET AL. Accidents cardiaques observés au cours des traitements par la rubidomycine. [Cardiac complications observed during treatment with rubidomycin.] Pathol Biol 15:949–953, 1967.
36. BONADONNA, G., and MONFARDINI, S. Letter to the Editor: Cardiac toxicity of daunorubicin. Lancet 1(7599):837, 1969.
37. RIPAULT, J., WEIL, M., and JACQUILLAT, C. Etude nécropsique de quatre malades traités par la rubidomycine. [Post-mortem study of four patients treated with rubidomycin.] Pathol Biol 15:955–957, 1967.
38. WANG, J., CORTES, E., SINKS, L., ET AL. Therapeutic effect and toxicity of adriamycin in patients with neoplastic disease. Amer Soc Clin Oncol 7:54, 1971.
39. MATHÉ, G., AMIEL, J.-L., HAYAT, M., ET AL. Essai de l'adriamycine dans le traitement des leucémies aiguës. Presse Med 78:1997–1999, 1970.

Effect of Concomitant Drug Treatment on Toxic and Therapeutic Activity of 5-Fluorouracil (5-FU; NSC-19893) [1,2]

C. G. Moertel, R. J. Reitemeier, and R. G. Hahn [3]

SUMMARY

The effects of concomitant drug therapy were evaluated by the double-blind method in 250 patients receiving 5-fluorouracil (5-FU) for the treatment of gastrointestinal cancer. Compared to placebo, the following drugs did not produce any significant increase in 5-FU toxicity or decrease in therapeutic effect: thiopropazate, prochlorperazine, pipamazine, thiethylperazine, chlorprothixene, sodium pentobarbital, cinnarizine, and trimethobenzamide.

[Cancer Chemother Rep 56:245–247, 1972]

The patient with advanced cancer who is selected for treatment with an antineoplastic agent frequently will have significant symptoms related to his cancer that also demand pharmacologic palliation. For the management of pain, salicylates, para-aminophenol derivatives, narcotics, or various synthetic anodynes may be required; for control of nausea and vomiting, antiemetic drugs may be required; for control of fluid retention, thiazide diuretics, spironolactone, triampterene, furosemide, or ethacrynic acid may be required; for relief of insomnia, barbiturates or other sedatives may be required; and for management of emotional disturbances, a variety of psychotherapeutic agents may be required. Concomitant drug treatment is, therefore, common among those patients selected for a trial of therapy with cytotoxic drugs; moreover, the side effects of chemotherapy may also require the use of additional pharmacologic agents.

The magnitude of this problem was impressed upon us by a review of our recent phase II studies of new drugs for the treatment of gastrointestinal cancer. In these studies, 85% of our patients received an average of four additional individual drugs (in either combined or pure form) for symptomatic and supportive purposes, either immediately prior to or during chemotherapy. Thus, the vast majority of our study patients were not receiving treatment with a single chemotherapeutic agent; most patients were in fact receiving combination chemotherapy. Each of the supportive drugs given was capable of inducing physiologic changes that could conceivably significantly alter either the toxicity or the therapeutic activity of the chemotherapeutic agent under investigation.

The purpose of the study reported in this paper was to evaluate, in retrospect, the effects of a number of symptomatic and supportive drugs on the toxicity and antineoplastic activity of 5-fluorouracil (5-FU).

MATERIALS AND METHODS

The data for this report were derived from two previous studies that were conducted to evaluate the antiemetic activity of a number of agents administered prophylactically to patients receiving 5-FU therapy (1,2). Both studies were prospective, randomized, and

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[2] 5-Fluorouracil was supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, National Cancer Institute.

[3] Division of Gastroenterology and Internal Medicine (Drs. Moertel and Reitemeier), and Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Clinic and Mayo Foundation, Rochester, Minn.

TABLE 1.—Concomitant drug therapy

Generic name	Proprietary name	Dose (mg)
Thiopropazate	Dartal	10
Prochlorperazine	Compazine	5
Thiethylperazine	Torecan	10
Pipamazine	Mornidine	10
Chlorprothixene	Taractan	50
Trimethobenzamide	Tigan	200
Cinnarizine	—	10
Sodium pentobarbital	—	30

double-blind in design; they were, however, planned only for the purpose of evaluating antiemetic activity. Patients were not selected or randomized for the purpose of obtaining a proper evaluation of 5-FU toxicity or therapeutic effect. Also, when patients receiving the study drug had nausea with repeated vomiting or experienced intolerable sedation, they were declared treatment failures and treatment with a known antiemetic, usually thiopropazate or prochlorperazine, frequently was substituted. The retrospective and selective nature of our present study must therefore qualify the validity of our results and conclusions.

From our initial studies we selected for evaluation (in the present study) only those patients who had gastrointestinal adenocarcinoma with measurable areas of known cancer to serve as objective indicators of response to therapy. Patients for whom treatment with a known antiemetic agent had been substituted during the course of therapy were excluded from evaluation, as were patients for whom followup data were inadequate with regard to toxic reactions and therapeutic response.

The 250 patients selected in this manner for evaluation all had received 5-FU by the standard rapid injection, intensive course method (ie, at a dose of 15 mg/kg/day for 5 days followed by a dose of 7.5 mg/kg on alternate days for four doses unless specific toxicity became evident). Two thirds of these patients had adenocarcinoma of the large bowel, and the remaining patients had gastrointestinal adenocarcinomas primary to a number of other sites, eg, stomach, pancreas, small bowel, liver (hepatoma). Over 90% of the patients had not received previous treatment with cytotoxic drugs. Leukocyte counts were obtained at least twice weekly for 3 weeks after the start of therapy, and indicator lesions were calibrated at 5 weeks.

The specific antiemetic drugs studied and the doses given are listed in table 1. All antiemetic drugs were administered orally three times daily (before meals) for the first 6 days of 5-FU therapy. Patients were advised not to take any other antiemetics, sedatives, or tranquilizers, but they were permitted to take other drugs, most frequently analgesics or diuretics. All study drugs and placebo were administered in identical opaque capsules. Neither patient nor physician was aware of their identity. USP lactose was used as placebo and as filler for all active agents.

TABLE 2.—Influence of concomitant drug therapy on toxicity and therapeutic effects of 5-FU treatment

Concomitant therapy	No. of patients treated	Toxic effects (% of patients treated)				Objective response (% of patients treated)		
				Leukopenia (cells/mm³)				
		Stomatitis	Diarrhea	<4000	<2000	Improved	Stable	Worse
Placebo (USP lactose)	44	57	43	74	26	18	52	30
Thiopropazate	43	63	19*	70	28	37*	37	26
Prochlorperazine	22	64	45	85	30	20	35	45
Thiethylperazine	18	39	39	67	33	33	39	28
Pipamazine	27	63	22	72	36	12	64	24
Chlorprothixene	11	64	18	73	18	18	27	55
Trimethobenzamide	23	83	43	73	32	20	65	15
Cinnarizine	28	75	15*	75	32	20	44	36
Sodium pentobarbital	24	42	42	71	21	26	30	44

* Variance from placebo at $P < 0.05$ level.

RESULTS

The toxicity and objective therapeutic results noted 5 weeks after the start of therapy are documented in table 2. This data is a composite of data from our two earlier studies that were selected for the present study according to the criteria listed above. Our criteria for objective response were (*a*) a reduction by at least 50% of the product of the longest perpendicular diameters of the most clearly measurable area of known cancer; (*b*) no increase in any other area of cancer; and (*c*) no development of any new areas of cancer.

There is no evidence that, when compared to placebo, any of the study drugs significantly added to 5-FU toxicity or significantly detracted from its therapeutic efficacy.

DISCUSSION

Although this study has admitted limitations in design, it seems unlikely that these limitations would produce any gross distortion in the results obtained. Concomitant administration of the phenothiazines (thiopropazate, prochlorperazine, pipamazine, and thiethylperazine), the phenothiazine relative (chlorprothixene), the barbiturate (sodium pentobarbital), the antihistamine (cinnarizine), and trimethobenzamide did not appear to produce any detectable adverse alteration in the clinical pharmacology of 5-FU.

Thiopropazate and cinnarizine were associated with less frequent diarrhea, and thiopropazate was associated with an increased objective response rate, all at the $P < 0.05$ level; these differences, however, must be interpreted very cautiously. When 64 different parameters of drug treatment are compared with those of placebo treatment, chance alone would allow that three parameters of drug treatment would differ from those of placebo treatment at a $P < 0.05$ level of significance.

It must be emphasized that these results, although reassuring in nature, pertain to only a single chemotherapeutic agent and to only eight of the multitude of drugs that might be given to manage symptoms or to treat unassociated diseases in the patient with cancer.

REFERENCES

1. MOERTEL, C. G., and REITEMEIER, R. J. Controlled clinical studies of orally administered antiemetic drugs. Gastroenterology 57:262–268, 1969.

2. MOERTEL, C. G., REITEMEIER, R. J., and GAGE, R. P. A controlled clinical evaluation of antiemetic drugs. JAMA 186:116–118, 1963.

Preliminary Clinical Trials of Dichloromethotrexate (NSC-29630) in Hepatocellular Carcinoma [1,2,3]

Charles L. Vogel, Richard H. Adamson, Vincent T. DeVita, David G. Johns, and Sebastian K. Kyalwazi [4,5]

SUMMARY

Sixteen patients with biopsy-proved hepatocellular carcinoma were treated with dichloromethotrexate. Although objective antitumor responses were not seen in patients receiving the drug intravenously, one significant objective response and one long-term survival (> 1 year) were noted in patients treated via hepatic artery catheters. The drug was less toxic in patients who did not have concomitant cirrhosis of the liver than in those patients whose tumors were superimposed on cirrhotic liver. The data suggest that expanded therapeutic trials of dichloromethotrexate administered via hepatic artery catheters are indicated.

[Cancer Chemother Rep 56:249–258, 1972]

Although there have been a number of reports on the chemotherapy for hepatocellular carcinoma, most of these reports describe results in small numbers of patients. In published studies of larger series of patients with this disease, attempts at systemic chemotherapy have been disappointing (1, 2). In contrast, some encouraging results have been reported with the use of cytotoxic agents administered via hepatic artery catheters (1–6).

On the basis of results of studies on transplantable liver tumors in animals, Adamson, Loo, and Morris (7) suggested that clinical trials with another folic acid antagonist, dichloromethotrexate (fig 1), might be of particular interest in the treatment of hepatocellular carcinoma. Dichloromethotrexate is rapidly converted by normal liver cells to an inactive metabolite (fig 1), and certain undifferentiated rat liver cell tumors were found to be unable to detoxify the drug. In addition, dichloromethotrexate is concentrated in the liver to a greater extent than methotrexate[6] when the two drugs are compared at equimolar dose levels (8).

The purpose of this report is to present preliminary results of systemic and intrahepatic arterial therapy with dichloromethotrexate for patients with hepatocellular carcinoma and to

[1] Received July 28, 1971; revised Jan 19, 1972.

[2] Supported by contract PH43-67-1343 from Chemotherapy, National Cancer Institute (NCI), National Institutes of Health.

[3] Dichloromethotrexate: glutamic acid, *N*-[3,5-dichloro-4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-. Supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, NCI.

[4] Solid Tumor Center of the Uganda Cancer Institute and Department of Surgery, Makerere University Medical School, Kampala, Uganda (Drs. Vogel and Kyalwazi); and the Solid Tumor Service (Dr. DeVita) and Laboratory of Chemical Pharmacology (Drs. Adamson and Johns), Chemotherapy, NCI, Bethesda, Md.

[5] The authors wish to acknowledge the efforts of the entire hepatocellular carcinoma "team" of the Uganda Cancer Institute including: Mr. D. Bhana, Mr. N. Harrison, and Dr. D. Dhru (Surgeons), Drs. D. Truscott and P. Briers (Radiologists), Dr. M. Dodson (Anesthesiologist), Dr. N. Mody and Mrs. I. Patel (alpha fetoprotein determinations), and Dr. V. K. Alpidovsky (Hematologist). Miss Z. Dhanani and Miss K. Pradhan helped with the biochemical tests for aldehyde oxidase. We thank also Dr. Allen Linsell and the International Agency for Research on Cancer for additional financial support. Appreciation is expressed also to all the physicians and surgeons throughout Uganda who referred patients to us for evaluation and therapy.

[6] Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-.

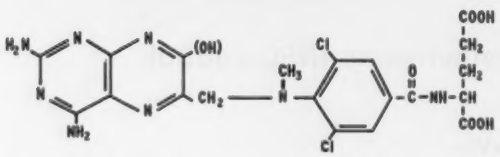


FIGURE 1.—Structure of dichloromethotrexate and its inactive 7-OH metabolite (3',5'-dichloro-4-amino-4-deoxy-7-hydroxy-N^{10}-methylpteroylglutamic acid).

discuss attempts at assaying tumor and non-tumorous liver from patients with this disease for the enzyme dichloromethotrexate oxidase (aldehyde oxidase; EC 1.2.3.1.) (9).

PATIENTS AND METHODS

Sixteen patients with biopsy-proved hepatocellular carcinoma were entered into the study while they were hospitalized at the Solid Tumor Center of the Uganda Cancer Institute. The Center is a 16-bed clinical research unit affiliated jointly with Makerere University Medical School in Kampala, Uganda, and the National Cancer Institute, Bethesda, Maryland. Only patients who had an expected survival time of greater than 1 month (untreated) were accepted for study. Informed consent, in accord with the principles promulgated by the World Medical Association as the Declaration of Helsinki, was obtained from each patient prior to the start of treatment. The treatment protocol was approved by the clinical research committees of the Uganda Cancer Institute, the Uganda National Research Council, and the National Cancer Institute (USA).

Pretreatment clinical evaluation included: complete history and physical examination; qualitative tests for alpha fetoprotein (AFP) (10); determination of hemoglobin, proline hydroxylase (11), urea, bilirubin (total and direct), alkaline phosphatase, and serum glutamic oxaloacetic transaminase (SGOT) levels; total and differential white blood cell count (wbc) and platelet count; prothrombin time; protein electrophoresis and immunoelectrophoresis. Roentgenograms of the chest, skull, pelvis, and lumbar spine, and colloidal gold liver scans were performed routinely. Complete blood cell counts and measurements of evaluable tumors were done at least weekly while patients were receiving treatment. Tests for AFP, urea, proline hydroxylase, and liver function were done serially in most patients.

All patients were staged retrospectively according to criteria established at an International Symposium on Hepatocellular Carcinoma (12) and all but two patients had functional stage II disease. A summary of patient characteristics appears in table 1. Ten of the 16 patients were men and six were women. The sera of 11 of the 16 (69%) were positive for AFP. The presence or absence of cirrhosis could not be assessed in four patients whose biopsy specimens contained no non-tumorous liver. Of the remaining 12 patients, seven had cirrhosis and five did not. None of the patients entered into the study had histologically well differentiated tumors: all tumors were either moderately or poorly differentiated.

All but three of the patients had not been treated previously; two of the three patients who had been treated (Nos. 18 and 123) had functional stage I disease and had undergone partial hepatectomy. Patient No. 18 was treated because a 5-mm tumor nodule was noted in the residual left lobe of his liver at the time of surgery; patient No. 123 received dichloromethotrexate because of persistent tenderness and increase in size of the residual right lobe of the liver and a persistently positive AFP test. The third previously treated patient (No. 91) had received a combination of BCNU and 5-fluorouracil without effect.[7] All patients, with the exception of the two who had received treatment after partial hepatic resection, had measurable tumors at the start of therapy.

Only two patients (Nos. 34 and 55) had mild (< 3.0 mg/100 ml) elevations in their total serum bilirubin levels and neither of these patients had clinically apparent jaundice.

Drug Dosage and Schedules

The maximal tolerated single daily parenteral dose of 75 mg/m²/day (13) was given as a 24-hour infusion in 5% dextrose and water to

[7] BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.
5-Fluorouracil: NSC-19893.

TABLE 1.—Patient characteristics and response to dichloromethotrexate therapy

Patient No. (age, sex)	Alpha feto-protein*	Tumor differen-tiation	Cirrhosis†	Measurable tumor	Proline hydroxylase (normal, <570 dpm/ml/hr)‡	Alkaline phosphatase (normal, 0–35 IU)	SGOT (normal, 0–36 IU)	Dichloro-methotrexate (length of infusion and route)§	Time to tumor progression (days)	Subsequent course¶
5 (40, F)	+	Poor	Absent	Liver, lung	460	62	63	24 hr (iv)	56	Died
8 (40, M)	+	Moderate	Present	Liver	ND	50	53	24 hr (iv)	14	Died
18 (18, M)	+	Moderate	Absent	None	518	76	72	4 hr (iv)	170	Alive with disease
34 (38, M)	−	Moderate	Present	Liver	557	192	172	4 hr (iv)	63	Died
37 (59, M)	+	Poor	Absent	Liver	993	30	48	4 hr (iv)	66	Surgery; died
48 (55, F)	+	Poor	Present	Liver	2902	180	98	4 hr (iv)	34	Died
55 (40, M)	+	Moderate	Present	Liver	3175	63	148	4 hr (iv)	26	Died
62 (26, F)	+	Poor	Present	Liver	532	48	150	24 hr (ia)	600	See text
63 (24, M)	+	Poor	Absent	Liver	817	46	120	24 hr (ia)	30	Died
91 (16, M)	+	Poor	NE	Liver, chest wall	523	234	75	4 hr (iv)	30	Received NSC–45388; died
123 (40, F)	+	Poor	Present	Persistent AFP	415	58	56	4 hr (iv)	60+	Alive with disease
149 (39, F)	−	Moderate	Absent	Liver	4748	110	120	4 hr (iv)	59	CCNU; died
160 (32, M)	+	Moderate	NE	Liver, lung	376	42	72	4 hr (iv)	30	CCNU; died
180 (40, M)	−	Poor	Present	Liver	3774	36	96	24 hr (ia)	66	See text
187 (68, M)	−	Moderate	NE	Liver	462	357	112	4 hr (iv)	30+	Alive with disease
189 (40, F)	−	Poor	NE	Liver	1622	72	280	4 hr (iv)	90	Died

*(+) assay positive for AFP; (−) assay negative for AFP.
†NE = not evaluable.
‡ND = not determined.
§Iv = intravenous; ia = intra-arterial.
¶ NSC–45388: imidazole–4–carboxamide, 5–(3,3–dimethyl–1–triazeno)–.
CCNU: NSC–79037; urea, 1–(2–chloroethyl)–3–cyclohexyl–1–nitroso–.

TABLE 2.—Toxic effects of dichloromethotrexate (4-hour infusions)

Patient No.	Course	Daily dosage (mg/m²)	Therapy completed (days)	Gastrointestinal toxicity*	Wbc count (×10³/mm³)			Platelet count (×10³/mm³)		
					Initial	Nadir (day)†	Recovery day	Initial	Nadir (day)	Recovery day
5	1	3.5	10	NT	—	NT	NT	—	NT	NT
	2	10	10	NT	—	NT	NT	—	NT	NT
18	1	5	10	NT	—	NT	NT	—	NT	NT
	2	12	10	++Diarrhea	—	NT	NT	—	NT	NT
	3	25	10	+++Diarrhea	—	NT	NT	—	NT	NT
	4	25	10	++Diarrhea	—	NT	NT	—	NT	NT
	5	25	10	+Diarrhea	—	NT	NT	—	NT	NT
34	1	28	9	++Diarrhea	4.1	2.2 (9)	15	170	54 (17)	19
	2	20	6	++Diarrhea, ++Stomatitis	6.6	3.7 (10)	12	140	52 (11)	—
37	1	15	10	NT	—	NT	NT	—	NT	NT
	2	20	10	NT	4.5	3.6 (14)	17	NT	NT	NT
	3	30	8	NT	5.9	2.1 (13)	17	340	80 (15)	17
48	1	25	5	+++Stomatitis, +Diarrhea	12.4	1.1 (12)	14	390	52 (12)	14
55	1	25	7	+++Diarrhea	16.4	2.7 (22)	—	NT	NT	NT
91	1	20	10	++Diarrhea	10.0	4.4 (9)	13	380	117 (13)	21
123	1	25	7	+Nausea	2.7	NE	NE	152	95 (13)	18
149	1	20	10	NT	—	NT	NT	—	NT	NT
	2	30	10	NT	—	NT	NT	—	NT	NT
160	1	25	6	NT	4.2	2.4 (6)	13	NT	NT	NT
	2	20	10	NT	4.8	3.0 (9)	21	NT	NT	NT
187	1	25	7	++Diarrhea	4.6	2.4 (10)	NE	NT	NT	NT
189	1	25	10	++Diarrhea	5.3	1.8 (17)	NE	170	42 (16)	22

*NT=no toxicity; +=mild; ++=moderate; +++=severe.
†NE=not evaluable.

patient No. 5, one of the first two patients studied; the other patient (No. 8) was thought to have underlying cirrhosis and was given approximately one quarter of that dose.

The drug was given to subsequent patients intravenously (iv) in 4-hour infusions daily for 10 days at doses ranging from 3.5 to 30 mg/m²/day. Administration of the drug was discontinued in the event of significant hematologic or gastrointestinal toxicity. If neither toxicity nor antitumor response was noted at one dose level, the next infusion in the same, or subsequent patient, was begun at a dose at least 25% higher than the previous dose. The doses, number of courses, and number of patients at each level are shown in table 2. Three patients who had tumor involvement of both lobes of the liver received dichloromethotrexate at a dose of 8–10 mg/m²/day (14) by continuous infusion through surgically implanted hepatic arterial catheters.

Qualitative and Quantitative Testing for Aldehyde Oxidase

Tumor and normal liver were obtained at laparotomy when this procedure was indicated for the purpose of diagnosis or therapy. Other specimens were obtained at postmortem examination performed within 4 hours of the death of a patient. All specimens were stored frozen at –20 C and were processed within 1 month.

Qualitative assays for aldehyde oxidase were performed essentially as described by Loo and Adamson (15). Various aspects of this basic procedure were altered in an effort to reproduce the characteristic spectral shift from 370μ to 352μ (denoting the oxidation of dichloromethotrexate) that was described by Adamson and Loo (16) and observed by us in similar studies using rat liver homogenates. Factors that were varied included: pH, substrate concentration, amount and concentration of homogenate, use of soluble homogenate fractions or boiled homogenates, and time of incubation. In addition, several attempts were made using fresh normal liver.

As an alternative method, dichloromethotrexate was incubated with liver homogenates, and the rate of enzymic inactivation of the drug was determined by measurement of residual dichloromethotrexate levels by the dihydrofolate reductase titration procedure of Bertino et al (17).

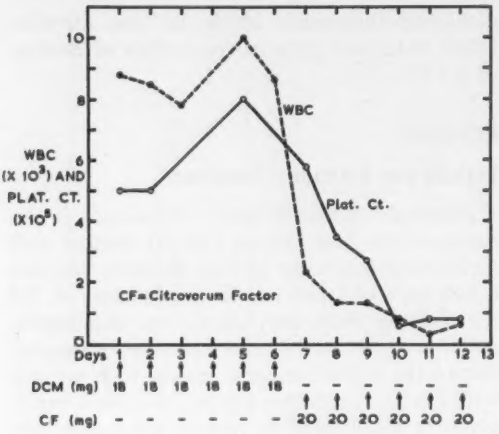
RESULTS

Toxicity and Antitumor Response

Systemic chemotherapy: continuous iv infusion.—The first patient (No. 8) treated with dichloromethotrexate by this schedule received 6 full days of treatment to a total dose of 108 mg. On the third day, stomatitis, esophagitis, and diarrhea occurred and increased in severity during the following days. In spite of treatment with citrovorum factor (20 mg/day for 6 days), given in an attempt to reverse the severe toxicity of the drug, the patient remained leukopenic and died 14 days after the start of treatment with dichloromethotrexate. His death was due to thrombocytopenia and intra-abdominal hemorrhage. (A summary of hematologic toxic effect appears in fig 2.)

The second patient (No. 5) was known to have no underlying cirrhosis and treatment with dichloromethotrexate at a dose of 75 mg/m²/day was begun. The severe toxicity of the drug encountered by patient No. 8 was noted during the second day of therapy in patient No. 5 and the drug was discontinued after a total dose of 218 mg had been given. Although this patient became leukopenic and severe mucositis developed, she recovered from these toxic effects without serious complications. (A summary of observed hematologic toxic effects appears in fig 3.) The patient subsequently received two additional courses of dichloromethotrexate administered iv in 4-hour infusions at doses of 3.5 and 10 mg/m²/day given for 10 days (to total doses of 52.5 and 130 mg respectively). There was no evidence of drug toxicity or patient response. She died of progressive tumor 56 days after the start of dichloromethotrexate therapy.

Systemic chemotherapy: 4-hour iv infusions.—Twenty-two courses of dichloromethotrexate were given to 12 patients treated with this dosage schedule at doses ranging from 3.5 to 30 mg/m². One of these patients (No. 5) previously had received continuous 24-hour infusions as described in the preceding section. A sum-


WBC
(X 10³) AND
PLAT. CT.
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WBC
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CF=Citrovorum Factor
Days 1 2 3 4 5 6 7 8 9 10 11 12 13
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FIGURE 2.—Hematologic toxicity in patient No. 8 who received continuous iv infusions of dichloromethotrexate.

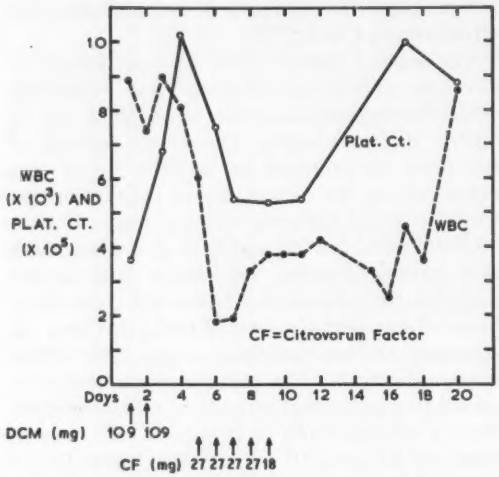

FIGURE 3.—Hematologic toxicity in patient No. 5 who received continuous iv infusions of dichloromethotrexate.

mary of hematologic and gastrointestinal toxic effects of the drug appears in table 2. Nadir leukocyte counts were encountered, on the average, 12 days after the start of treatment. Severe leukopenia (total wbc $< 2000/mm^3$) was encountered in two patients, both of whom had concomitant cirrhosis. No serious infectious complications occurred in any patients. Day 14 was the mean day of nadir platelet count in patients with thrombocytopenia; however, no hemorrhagic complications attributable to drug-induced thrombocytopenia were encountered. The drug was less toxic in patients Nos. 18, 37, and 149, whose hepatocellular carcinomas were superimposed on normal (non-cirrhotic) liver, than in cirrhotic patients receiving similar doses of dichloromethotrexate.

Most patients treated according to this schedule had objective evidence of tumor progression while they were receiving treatment (table 1); in no patient was an objective antitumor effect seen. The case of patient No. 18 is especially instructive since dichloromethotrexate was administered when the tumor had been surgically reduced to a minimum prior to chemotherapy: the drug was unable to prevent the development of recurrent tumor.

Intrahepatic arterial chemotherapy: continuous infusion.—Three patients received dichloromethotrexate intra-arterially via surgically implanted hepatic artery catheters. The first patient (No. 62) received dichloromethotrexate for a total of 8 days (to a total dose of 38 mg). The catheter was removed on Day 14 when a catheter angiogram revealed that most of the blood flow was to the massively enlarged spleen. Splenomegaly in this patient was thought to be due to the tropical splenomegaly syndrome (18). Intrahepatic arterial dichloromethotrexate therapy was complicated by diarrhea beginning on the third day of treatment and recurring on each subsequent day of

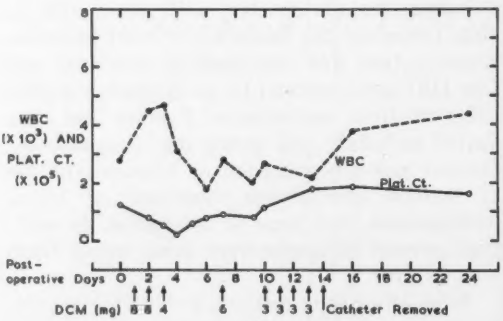

FIGURE 4.—Hematologic toxicity in patient No. 62 who received continuous infusions of dichloromethotrexate via an hepatic artery catheter.

dichloromethotrexate administration. Alterations in wbc and platelet counts during therapy are presented in figure 4. Interpretation of these changes, however, is complicated by the fact that the effects observed could have been related to (or caused by) either the drug or hypersplenism. The patient died of recurrent disease 21 months after the completion of intrahepatic arterial dichloromethotrexate therapy.

Patient No. 63 received dichloromethotrexate treatment for 3 days at a dose of 6 mg/m²/day. On the last day of treatment, diarrhea occurred and persisted for the next 3 days, and his platelet count dropped from a pretreatment value of 370,000 to 100,000 cells/mm³. The platelet count returned to normal 4 days later and leukopenia never occurred. A catheter angiogram revealed that most of the blood flow was to the spleen and no further therapy was given, although the catheter remained in situ in the hepatic artery until the patient's death 1 month later. At postmortem examination, the catheter was located in the hepatic artery but the tip was directed toward the splenic artery orifice.

Patient No. 180 received dichloromethotrexate at a dose of 7.5 mg/m²/day for 5 days. The patient had diarrhea on the fourth and fifth days of treatment. Hematologic toxic effects in this patient are summarized in figure 5. Within 1 month after the termination of dichloromethotrexate therapy, there was a striking decrease in the size of both the liver and the discrete, palpable tumor nodule. Concomitant with the objective evidence of tumor regression, shown in figure 6, there was a dramatic decrease in serial proline hydroxylase levels from 3774 dpm/ml/hr on admission to normal values (< 570 dpm/ml/hr) 3 weeks after the drug was discontinued. Subsequently, a subphrenic abscess occurred and precluded administration of cytotoxic chemotherapy for the next 3 months. Four months after the first course of dichloromethotrexate, the patient had a tumor recurrence which extended through the surgical scar onto the anterior abdominal wall. A second course of the drug (to a total dose of 110 mg), administered at this time via a percutaneously implanted hepatic artery catheter, also resulted in a greater than 50% reduction in the size of visible tumor; however, the patient soon had recurrent tumor that was refractory to further dichloromethotrexate therapy, and he died 9 months from the start of therapy.

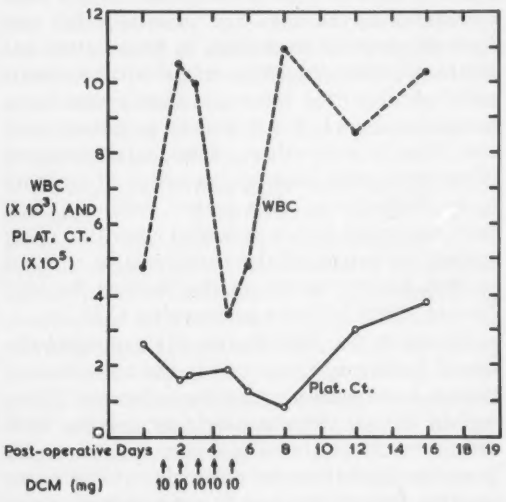

FIGURE 5.—Hematologic toxicity in patient No. 180 who received continuous infusions of dichloromethotrexate via an hepatic artery catheter.

Liver Function Tests

Pretreatment alkaline phosphatase and SGOT levels are summarized in table 1. No significant improvement in the abnormal results of liver function tests was noted in any patient receiving any form of iv or intra-arterial dichloromethotrexate therapy. Increasingly abnormal results of these tests were frequently encountered but, in general, they were thought to be secondary to progressive tumor and not to drug toxicity.

In Vitro Assays for Aldehyde Oxidase

Specimens of tumor from nine patients and non-tumorous liver from seven of these same patients were available for in vitro assay. Three of the non-tumorous liver specimens were cirrhotic and four were histologically normal. In addition, normal liver specimens obtained post mortem from three control patients with no evidence of liver disease were tested. In no instance was the characteristic ultraviolet

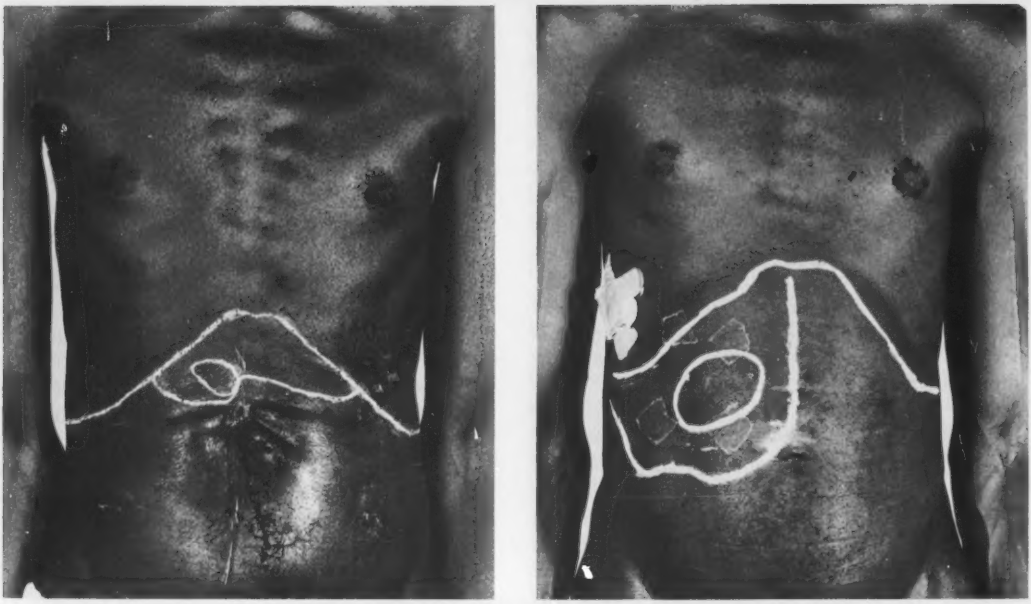

FIGURE 6.—Patient No. 180: *left*—pretreatment clinical photograph; *right*—photograph taken 3 months later.

spectral shift from 370μ to 352μ observed in the qualitative test. None of the alterations in the in the basic assay procedure listed previously resulted in the spectral shift denoting dichloromethotrexate oxidation. Similarly, measurement of the rate of oxidation of dichloromethotrexate by the dihydrofolate reductase inhibition assay showed that while rapid metabolism and inactivation of the drug occurred with control rat liver homogenates, metabolism could not be detected with either the normal or the neoplastic human liver tissue.

DISCUSSION

Hepatocellular carcinoma is a relatively rare tumor in the Western world but it is a significant clinical problem in many parts of Africa and the Far East. In these areas where the disease is endemic, it is often far advanced when first diagnosed and generally pursues a rapidly progressing clinical course (1, 2, 19). Occasionally, patients with localized disease can be benefited by partial hepatic resection or by the use of antimetabolites administered via hepatic artery catheters (1–6); however, most patients have far-advanced disease at the time of diagnosis, and thus are unsuitable for any form of surgical treatment. In these latter patients, chemotherapeutic trials with systemically administered cytotoxic agents have been disappointing (1, 2). It should be noted, however, that in most other studies, small numbers of patients were used, and selection of patients to be studied was often poorly defined. It has been suggested that a universal clinical staging system for hepatocellular carcinoma be adopted so that future reports on the therapy for this disease might be more comparable (12).

Adamson, Loo, and Morris (7) suggested the use of dichloromethotrexate in the treatment of human hepatocellular carcinoma because it has certain unique pharmacologic properties. This drug accumulates in normal liver (8), is rapidly metabolized there by aldehyde oxidase to an inactive form (16), and is not metabolized by the "multiple deviation" Novikoff hepatoma in rodents (7). They reasoned that if the drug

was administered to patients whose liver tumors could not metabolize dichloromethotrexate, a high therapeutic index might be anticipated. The present study was designed to assess the therapeutic efficacy of dichloromethotrexate in the treatment of human hepatocellular carcinoma and to attempt to correlate clinical response with the results of in vitro tests for aldehyde oxidase in tumor tissue.

The results of these preliminary clinical trials with 4-hour iv infusions of dichloromethotrexate in the treatment of hepatocellular carcinoma were disappointing. Although it is still possible that iv administered dichloromethotrexate given according to some other schedule might prove effective, definite evidence of tumor regression was not encountered in any patient treated iv with dichloromethotrexate in the present series. In most of our patients there was evidence of tumor progression during treatment and in one patient, tumor progression occurred even though dichloromethotrexate treatment was begun only after the tumor had been surgically reduced to a minimum. Drug toxicity, manifested by mucositis, diarrhea, and hematologic depression, was generally more severe in patients whose tumors were superimposed on cirrhosis than in those whose residual liver tissue was histologically normal. A similar observation of more severe dichloromethotrexate toxicity in patients with cirrhosis than in patients with normal liver function has been reported by Cleveland et al (14) in a study on the use of this drug in the treatment of cancer of the head and neck area.

Several possibilities exist to explain the poor response to iv administered dichloromethotrexate in this study. No information is available on either tumor cell membrane permeability to dichloromethotrexate, or active transport mechanism for the drug, either in vivo or in vitro. If dichloromethotrexate could not enter tumor cells adequately, any theoretical therapeutic advantage of the drug would be negated. The use of dichloromethotrexate in human hepatocellular carcinoma is also based on the assumption, as yet untested, that the observations of Adamson, Loo, and Morris (7) on aldehyde oxidase levels in minimal and multiple deviation rodent hepatomas could be correlated with the clinical response of such tumors to the drug. If such a correlation exists, the poor response to dichloromethotrexate therapy observed in the present series could be explained if most of the patients studied had tumors capable of rapidly detoxifying the drug.

In contrast to the results of iv therapy, both patients with evaluable responses treated with intrahepatic arterial doses of dichloromethotrexate appear to have benefited. Although the average survival time from the time of diagnosis in Ugandan patients with hepatocellular carcinoma is reported to be less than 2 months (19), these two patients survived for 9 and 21 months respectively. In addition, one of these patients had significant objective evidence of tumor regression after each of two courses of intrahepatic arterial doses of dichloromethotrexate. Both patients had minimal therapy (total doses of 38 and 50 mg respectively), and both had underlying cirrhosis. Although further studies will be necessary to determine whether the intra-arterial route of administration of dichloromethotrexate yields a therapeutic response significantly superior to that obtained by the iv route, such a difference, if reproducible, could most readily be explained by the attainment of a greater concentration of the drug in the tumor by the former route, before dilution by the body fluids.

Attempts were made to correlate the responses of the two patients having apparent responses to dichloromethotrexate treatment with histologic and biochemical parameters. Since studies in rodents appeared to demonstrate an inverse relationship between tumor enzyme content and histologic differentiation of the tumor, it might be expected that these two patients, with histologically poorly differentiated tumors, might have low levels of aldehyde oxidase.

Attempts at proving this latter point by biochemical assay procedures, however, were unsuccessful. The qualitative assay for dichloromethotrexate described by Loo and Adamson (15), although sensitive enough to detect substantial enzyme activity in rodent liver, was not satisfactory for use with human material. In the qualitative test, the characteristic ultraviolet spectral shift from 370μ to 352μ, denoting

oxidation of dichloromethotrexate to its inactive metabolite 3′,5′-dichloro-4-amino-4-deoxy-7-hydroxy-N^{10}-methylpteroylglutamic acid (fig 1) observed after incubation of dichloromethotrexate with rodent liver, was not observed with any of the nine tumor specimens or ten cirrhotic and normal liver specimens tested.

Indirect assays for the presence of the dichloromethotrexate oxidase enzyme (by measuring the amount of dihydrofolate reductase inhibition by the drug after incubation with human tumor and liver tissues) also did not detect any measurable oxidation of the drug even though in vivo studies have demonstrated that dichloromethotrexate does undergo partial metabolism in man (8). These findings might well be explained by the report of Cleveland et al (14), which indicated that the rate of oxidation of dichloromethotrexate by normal human liver is relatively slow (approximately 2% of that in rat liver, and 0.001% of that in guinea pig liver). It is possible, therefore, that the rate of oxidation of the drug by human liver tissue in vitro is too slow to be measured by the two methods used in the present study.[8] Alternatively, the human liver enzyme may have lost activity as a consequence of the freezing and thawing procedure to which most of the samples were subjected during storage. A third possibility is that the incubation conditions used may not have been optimal for the human liver enzyme. Further study will be necessary to distinguish between these possibilities; however, it is highly likely that our inability to detect enzyme activity in any liver or tumor specimen in the present study reflects a methodologic deficiency, and not a complete lack of enzyme capacity in the patients studied.

Although the results of treatment with 4-hour iv infusions of dichloromethotrexate were disappointing, the possible beneficial results obtained in two patients treated with intrahepatic arterial doses of dichloromethotrexate provide some encouragement for further approaches to treatment. Further studies using this route of drug administration are in progress.

[8] In liver biopsy samples obtained from a single patient with primary hepatocellular carcinoma (Johns, D. G., and Cleveland, J. C., unpublished data), measurable levels of dichloromethotrexate oxidase were detected in samples from non-tumorous areas, while the enzyme was undetectable in a biopsy from the tumor area. A method requiring preliminary enzyme purification procedures (9) prior to assay was used.

REFERENCES

1. GEDDES, E. W., and FALKSON, G. Malignant hepatomas in the Bantu. Cancer 25:1271–1278, 1970.
2. CHAN, K. T. The management of primary liver carcinoma. Ann R Coll Surg Engl 41:253–282, 1967.
3. PROVAN, J. L., STOKES, J. F., and EDWARDS, D. Hepatic artery infusion chemotherapy in hepatoma. Br Med J 3:346–349, 1968.
4. GORGUN, G., and WATNE, A. L. Infusion chemotherapy in hepatoma and metastatic liver tumors. Am J Surg 113:363–368, 1967.
5. LIN, T. Y. Primary cancer of the liver. Quadrennial review. Scand J Gastroenterol 5 (suppl 6): 223–241, 1970.
6. ARIEL, I. M., and PACK, G. T. Recent Results in Cancer Research: Tumors of the Liver. Heidelberg, Springer-Verlag, 1970, vol 26, pp 277–292.
7. ADAMSON, R. H., LOO, T. L., and MORRIS, H. P. Metabolism of $C1^{36}$-dichloromethotrexate by transplantable liver tumors. Proc Soc Exp Biol Med 111:566–568, 1962.
8. DAVIDSON, J. D., and OLIVERIO, V. T. The physiologic disposition of dichloromethotrexate-$C1^{36}$ in man. Clin Pharmacol Ther 6:321–327, 1965.
9. JOHNS, D. G. Human liver aldehyde oxidase: differential inhibition of oxidation of charged and uncharged substrates. J Clin Invest 46:1492–1505, 1967.
10. VOGEL, C. L., ANTHONY, P. P., MODY, N., ET AL. Hepatitis-associated antigen in Ugandan patients with hepatocellular carcinoma. Lancet 2 (7674): 621–624, 1970.
11. STEIN, H. D., KEISER, H. R., and SJOERDSMA, A. Proline-hydroxylase activity in human blood. Lancet 1 (7638):106–109, 1970.
12. VOGEL, C. L., and LINSELL, C. A. An international symposium on hepatocellular carcinoma. J Natl Cancer Inst 48:567–571, 1972.
13. FREI, E. III, SPURR, C. L. BRINDLEY, C. D., ET AL. Clinical studies of dichloromethotrexate (NSC 29630). Clin Pharmacol Ther 6:160–171, 1965.
14. CLEVELAND, J. C., JOHNS, D. G., FARNHAM, G., ET AL. Arterial infusion of dichloromethotrexate in cancer of the head and neck: a clinicopharmacologic study. *In* Current Topics in Surgical Research (Zuidema, G. D., and Skinner, D. B., eds). New York, Academic Press, 1969, vol 1, pp 113–120.
15. LOO, T. L., and ADAMSON, R. H. The enzymic oxidation of certain folic acid antagonists. Biochem Pharmacol 11:170–171, 1962.
16. LOO, T. L., and ADAMSON, R. H. The metabolite of 3′,5′-dichloro-4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (dichloromethotrexate). J Med Chem 8:513–515, 1965.
17. BERTINO, J. R., CASHMORE, A. R., and HILLCOAT, B. L. "Induction" of dihydrofolate reductase: purification and properties of the "induced" human erythrocyte and leukocyte enzyme and normal bone marrow enzyme. Cancer Res 30:2372–2378, 1970.
18. MARSDEN, P. D., and HAMILTON, P. J. Splenomegaly in the tropics. Br Med J 1:99–102, 1969.
19. ALPERT, M. E., HUTT, M. S., and DAVIDSON, C. S. Primary hepatoma in Uganda. A prospective clinical and epidemiological study of forty-six patients. Am J Med 46:794–802, 1969.

Phase II Study of 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) in the Treatment of Bronchogenic Carcinoma [1, 2]

Seymour Olshin, Saleem Siddiqui, and Dincer Firat [3,4]

SUMMARY

Twenty-five patients who had carcinoma of the lung were treated with intravenously administered 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) at a dose of 5 mg/kg body weight every 6 weeks. Two patients were lost to followup study and no objective responses were observed in the others. The toxicity was moderate and toxic reactions were mainly nausea, vomiting (within 1–2 hours of drug administration), and bone marrow depression (within 3–4 weeks). BCNU has proved ineffective in the treatment of bronchogenic carcinoma at the dosage and schedule used in this study.

[Cancer Chemother Rep 56:259–261, 1972]

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), a nitrosourea compound, has been found to suppress the growth of malignant tumors in both man and experimental animals (1–4). Evidence would indicate that the drug's action is similar to that of the biologic alkylating agents, although BCNU has been shown to inhibit several key enzymatic steps which ultimately lead to the formation of DNA.[5] Its effectiveness has been studied most extensively in acute leukemia; however, preliminary trials of BCNU in a variety of human cancers have indicated its possible effectiveness in the treatment of carcinoma of the lung (1,2).

The purpose of this study was to evaluate the effectiveness of BCNU in the treatment of a larger series of patients with either inoperable, recurrent, or metastatic bronchogenic carcinoma.

MATERIALS AND METHODS

This study comprised 25 patients who had histologically proved bronchogenic carcinoma with at least one measurable malignant lesion. The disease was confined to one lung in only three patients. The others had distant or contralateral metastases or both. There was a treatment-free observation period of at least 3 weeks for patients who had had either surgical treatment or radiotherapy prior to BCNU treatment. None of the patients had received chemotherapy with other antineoplastic agents prior to treatment.

The therapeutic regimen consisted of BCNU administered intravenously and repeated at 6-week intervals for 12–18 weeks. The dose was 5 mg/kg of body weight given in one injection. A pretreatment white blood cell count (wbc) of 5000/mm^3 and platelet count of 100,000/mm^3 was requisite for each patient. Second and third courses of therapy were modified by a 25% reduction in drug administered when the wbc dropped to less than 2000/mm^3 or the platelet count dropped to less than 50,000/mm^3 or

[1] Received Aug 4, 1971; revised Oct 21, 1971; accepted Nov 1, 1971.

[2] BCNU was supplied by Chemotherapy, National Cancer Institute.

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[4] *Reprint requests to:* Dincer Firat, MD, Department of Medicine, Long Island Jewish Medical Center-Queens Hospital Center Affiliation, 82–68 164th St, Jamaica, NY 11432.

[5] Broder, L. E., and Carter, S. K. Clinical brochure: BCNU, NSC-409962. Prepared by the National Cancer Institute, Bethesda, Md, revised Sept 1970, p3.

either count failed to return to pretreatment levels. If the wbc remained less than 4000/mm³ or the platelet count less than 75,000/mm³, the drug was witheld entirely pending recovery to these levels. Pretherapy baseline evaluation of the patient included chest roentgenogram; complete blood cell count; serum bilirubin, calcium, phosphorus, albumin, total protein, uric acid, alkaline phosphatase, lactic dehydrogenase, blood urea nitrogen, and glucose levels; urinalysis; and histologic diagnosis of tissue. Patients were seen at 1–3-week intervals for evaluation of drug toxicity and response to chemotherapy.

The results of chemotherapy were evaluated on the basis of objective tumor regression, using the following criteria:

Complete response—Complete disappearance of all measurable disease parameters
Partial (good)—Regression of measurable tumors by 50% or more
Partial (fair)—Regression of measurable tumors by 25%–50%
No response—Progression of the disease, no regression of the tumor, or a response less than 25%

RESULTS

Twenty-five patients received one to three courses of BCNU treatment. Only two patients were lost to followup study within 2 weeks of therapy and were excluded from the study. Most of the remaining 23 patients had either epidermoid or oat cell carcinoma (table 1). Eighteen of the 23 patients were men and five were women. The median ages were 57 years and 56 years, respectively.

Prior to treatment with BCNU, only two of the 23 patients had had radical surgical treatment with intent to cure. A third patient with limited disease was considered inoperable because he had advanced emphysema. Fourteen patients had received prior radiation therapy to their primary lesion, ranging from 5000 to 6000 rads tumor dose from a cobalt 60 radiation source, delivered in a 5–6-week period.

TABLE 1.—Histologic diagnosis of 23 patients receiving BCNU therapy

Histologic type	Number of patients	Male	Female
Epidermoid carcinoma	12	12 (2)*	—
Oat cell carcinoma	5	2	3
Anaplastic carcinoma	2	2 (1)	—
Adenocarcinoma	2	—	2 (1)
Papillary carcinoma	1	1	—
Bronchoalveolar carcinoma	1	1	—
Total	23	18	5

* Figures in parentheses indicate the number of patients who received only one course of therapy.

At the beginning of chemotherapy, disease limited to one lung was present in three patients; of these three, two had pleural effusion containing malignant cells. The remaining 20 patients had advanced carcinoma involving the opposite lung (seven patients), supraclavicular or axillary nodes (10 patients), or subcutaneous nodules (three patients). Four patients had liver metastases demonstrated by liver scans. Bone metastases were present in three patients, superior vena cava compression in two patients, and brain metastases in one patient.

Four of the 23 patients received only one course of BCNU therapy. Of these, one died on the 13th day after treatment. His wbc was 350/mm³ and sepsis had occurred. Iliac crest marrow aspiration 1 week after the treatment (6 days before the patient's death) had revealed hypocellularity but no evidence of tumor cells or fibrosis, although his pretreatment wbc counts and platelet counts were normal. Three other patients died of progressive disease 3, 17, and 25 days after the start of chemotherapy. Ten patients received three courses and nine patients received two courses of BCNU. In the latter patients, therapy was discontinued prior to the third course due to either the rapid progression of the disease or death.

The average time lapse from diagnosis to treatment with BCNU was 7 months. At the time of this paper, 18 of the 23 patients are dead and five are still alive with their disease. There was either no measurable tumor response or progression of the disease in all patients studied.

The median survival time for all patients was 10.2 months from the time of diagnosis, with 20% of the patients surviving 22.8 months or longer. On the other hand, only 50% of the patients with advanced disease were alive 4.2 months and 20% were alive 9.8 months from the start of chemotherapy. The median survival time of three patients with limited disease was 8.8 months from the start of BCNU therapy.

Toxic effects of the drug consisted of nausea and vomiting in seven patients within 1–3 hours. Depression of peripheral leukocyte and thrombocyte counts occurred in five patients; one died of agranulocytosis, and severe leukopenia (2000–3000/mm^3) and thrombocytopenia (75,000–100,000/mm^3) developed in four. Two additional patients had only mild leukopenia ranging between 4500/mm^3 and 5000/mm^3.

DISCUSSION

BCNU was reported to induce objective remissions in three of 28 patients with bronchogenic carcinoma studied in one series (2). On the other hand, initial reports of further clinical trial with this compound indicated no clear superiority of BCNU over placebo in prolongation of survival when compared to historical control groups (5).

In our series, no objective regression of the tumor was observed. The survival time from the start of chemotherapy for the two groups of patients with advanced disease (50% alive at 4.2 months and 20% alive at 9.8 months) is not significantly different from historical control groups, considering the wide variations in median survival frequently observed in such studies. Variations in the survival times of our patients with limited and advanced disease from those reported earlier by others (5) may well be due to such factors as patient selection, limited number of patients studied, and degree of effectiveness of previous treatments.

Although the present study includes a limited number of patients, the estimated rate of effectiveness of chemotherapy of bronchogenic carcinoma with BCNU as outlined here, would be low even in a larger series, since none of our patients benefited from the treatment (6). Therefore, based on our experience and that of others, we concluded that the further trial of BCNU in the treatment of lung cancer, at the dosage and schedule used in this study, is not warranted. However, the treatment regimen used in this study was moderately toxic and the effectiveness of BCNU therapy at higher dosage or in combination with other agents or modality of treatment could not be ruled out.

REFERENCES

1. DeVita, V. T., Gold, G. L., Owens, A. H., Jr., et al. Preliminary studies with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Proc Am Assoc Cancer Res 5:15, 1964.
2. DeVita, V. T., Carbone, P. P., Owens, A. H., Jr., et al. Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea, NSC-409962. Cancer Res 25:1876–1881, 1965.
3. Carter, S. K., and Newman, J. W. Nitrosoureas: 1,3-Bis(2-chloroethyl)-1-nitrosourea (NSC-409962; BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC-79037; CCNU)—clinical brochure. Cancer Chemother Rep Part 3, vol 1:115–151, 1968.
4. Hananian, J., Holland, J. F., and Sheehe, P. Intensive chemotherapy of acute lymphocytic leukemia in children. Proc Am Assoc Cancer Res 6:26, 1965.
5. Carbone, P. P. (moderator), Frost, J. K., Feinstein, A. R., et al. Lung cancer: perspectives and prospects.. Ann Intern Med 73:1003–1024, 1970.
6. Gehan, E. A. The determination of the number of patients required in a preliminary and follow-up trial of a new chemotherapeutic agent. J Chronic Dis 13:346–353, 1961.

Further Study of Trimethylcolchicinic Acid, Methyl Ether, *d*-Tartrate (TMCA; NSC-36354) in Patients With Malignant Melanoma[1, 2, 3]

David C. Stolinsky, Edwin M. Jacobs, Jack Braunwald, and Joseph R. Bateman [4,5]

SUMMARY

Fifty-six patients with disseminated malignant melanoma were treated with single daily oral doses of trimethylcolchicinic acid, methyl ether, *d*-tartrate (TMCA), an analog of colchicine (NSC-757). Response to therapy could be evaluated in 44 patients; objective responses occurred in four patients (9%). Toxic effects of therapy included leukopenia, thrombocytopenia, nausea, vomiting, stomatitis, alopecia, and dermatitis. When given according to this schedule, TMCA has not proved to be clinically useful in the treatment of malignant melanoma.

[Cancer Chemother Rep 56:263–265, 1972]

In a previous report (1) involving a trial of trimethylcolchicinic acid, methyl ether, *d*-tartrate (TMCA) in the treatment of 253 patients who had a variety of malignant neoplasms, objective responses were noted in four of 14 patients with malignant melanoma whose cases could be evaluated. On the basis of these data, the suggestion was made that further trial of TMCA treatment in patients with melanoma would be of interest. An extension of the previous study, limited to patients with melanoma, is described in the present report. Since the previous report, an additional 30 patients have been observed for response; no additional responses have been noted.

METHODS

Patient Selection

Patients who had histologically proved, measurable tumors not amenable to surgical treatment were included in the study. Patients who had had major surgery or radiation therapy within the prior 6 weeks were excluded from the study, as were those patients receiving other forms of antitumor therapy concurrently. Patients who had received other antitumor drugs in the prior 6 weeks were acceptable only if progression of their disease was apparent and if there were no residual toxic effects of the prior therapy.

Dose Schedule

Patients received orally administered single daily doses of TMCA before breakfast. The standard dose schedule was 0.05 mg/kg daily for the first week, 0.075 mg/kg daily for the second week, and 0.10 mg/kg daily for subsequent weeks. Patients manifesting severe

[1] Received Oct 19, 1971; accepted Dec 15, 1971.
[2] Supported in part by grant CA-05186 from the National Cancer Institute (NCI), National Institutes of Health, Public Health Service.
[3] Trimethylcolchicinic acid, methyl ether, *d*-tartrate; colchicine, *N*-deacetyl-, L-tartrate (1:1), hydrate; TMCA. The compound was supplied by Drug Research and Development (formerly Cancer Chemotherapy National Service Center), Chemotherapy, NCI.
[4] The following members of the Western Cooperative Cancer Chemotherapy Group participated in this study: *E. M. Jacobs, F. D. Johnson,* and *D. A. Wood,* Cancer Research Institute, University of California Medical Center, San Francisco; *D. C. Stolinsky, L. E. Irwin, G. J. Hum,* and *J. R. Bateman,* University of Southern California School of Medicine and John Wesley Hospital, Los Angeles; *J. Braunwald,* Kaiser Foundation Hospital, Los Angeles, Calif; *A. R. Stevens,* University of Washington School of Medicine, Seattle; *R. P. Pugh,* Ellis Fischel State Cancer Hospital, Columbia, Mo; *J. Solomon,* City of Hope Medical Center, Duarte, Calif.
[5] *Reprint requests to:* Dr. Stolinsky, University of Southern California Oncology Service, 2825 South Hope St, Los Angeles, Calif 90007.

cachexia or hypocellularity of the bone marrow were given initial doses of 0.025 mg/kg daily for the first 2 weeks; if no toxic effects were noted, the standard dose schedule was then begun. TMCA treatment was discontinued at the appearance of significant depression of the white blood cell count (wbc) or platelet count, at the first sign of stomatitis, or if the patient had significant nausea or vomiting. When the toxic effects cleared, treatment with TMCA was restarted at the previous dose or at a lower dose, at the discretion of the investigator.

Clinical Evaluation

Pretreatment examination of patients included: complete physical examination with bidimensional measurements of palpable tumors; complete blood cell count (platelets and reticulocytes included); urinalysis; determination of serum urea nitrogen or creatinine, alkaline phosphatase, bilirubin, and glutamic oxaloacetic transaminase levels; chest roentgenogram, and skeletal survey. Levels of serum albumin and globulin, as well as urinary melanin and melanogen, were determined also for many patients. Patients were seen at least weekly during therapy. Complete blood cell counts (platelets included) were obtained at least weekly. Liver and kidney function tests were repeated monthly, as were roentgenograms when abnormalities were noted.

RESULTS

Fifty-six patients (total) were treated with TMCA. One patient had received radiation therapy within 2 weeks prior to treatment with the drug and was therefore ineligible for inclusion in the study. In all of the remaining 55 patients, drug toxicity could be evaluated; however, response to treatment could not be evaluated in 11 of the 55 because they had completed fewer than 5 weeks of therapy. (As will be shown, the observed responses began within 5 weeks after the start of TMCA therapy.) Response to treatment could be evaluated in the remaining 44 patients. The number of patients in this group studied by each member institution of the Western Cooperative Cancer Chemotherapy Group is shown in table 1.

TABLE 1.—Number of patients with evaluable responses studied by member institutions*

Institution	No. of patients
University of California, San Francisco	29
University of Southern California	8
Kaiser Foundation, Los Angeles	5
University of Washington	1
Ellis Fischel State Cancer Hospital	1

*Western Cooperative Cancer Chemotherapy Group.

Responses

Criteria of response are outlined in table 2. Objective responses were observed in four of the 44 patients in whom response could be evaluated (9%); three of the responses were in the I-B category and one response was in the I-A category. The responses began on Days 14, 25, 29, and 35, respectively. The cumulative dose given to the start of response varied from 0.8 to 3.1 mg/kg. The responses lasted 4, 9, 15, and 59 months, respectively. Two additional patients had stabilization of previously progressive disease for 11 and 12 months, respectively, but these patients were not classified as objective responders. Lesions responding to therapy included those in skin, subcutaneous tissue, lymph nodes, and lung. One of the patients responding to TMCA had previously failed to respond to melphalan and mechlorethamine;[6] the other three patients responding to TMCA had not received previous chemotherapy.

Toxic Effects

The incidence and severity of toxic effects are outlined in table 3. Toxic effects were noted in 39 of the 55 patients in whom toxic effects could be evaluated. The onset of leukopenia occurred at a median of 29 days (range, 7–140 days) and at a median cumulative dose of 2.6 mg/kg (range, 0.2–12.8 mg/kg). The median duration of leukopenia was 7 days (range, 2–21 days). The figures for onset and duration of

[6] Melphalan; NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl) amino]phenyl]-, monohydrochloride, L-; L-phenylalanine mustard.
Mechlorethamine: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard.

TABLE 2.—Categories of response*

Category	Definition
0	No clinically useful effect on course of disease.
0–0	Disease progresses; no objective or subjective improvement.
0–A	Subjective improvement without objective changes.
0–B	Objective improvement for 1 month or more without subjective improvement.
0–C	Objective and subjective improvement of less than 1 month's duration.
I	Objective and subjective improvement of at least 1 month's duration.
I–A	Objective improvement of 25%–50% for 1 month or more, with subjective improvement
I–B	Objective improvement greater than 50% in all lesions for 1 month or more, with subjective improvement.
I–C	Complete objective and subjective remission for 1 month or more; regression of all manifestations of disease.
II	Interruption of progression of disease for 1 month or more.

*Modified from Karnofsky (2).

TABLE 3.—Toxic effects of TMCA in 55 patients

Toxic effect	No. of patients
Leukopenia (wbc/mm³)	
3000–3900	10
2000–2900	12
1000–1900	4
<1000	3
Total	29
Thrombocytopenia (platelets/mm³)	
100,000–149,000	5
50,000–99,000	3
30,000–49,000	1
<30,000	0
Total	9
Gastrointestinal	
Nausea	10
Vomiting	3
Stomatitis	8
Dermatologic	
Alopecia	9
Dermatitis	3
Depigmentation	1

thrombocytopenia were quite similar. One patient died of sepsis during a period of severe, drug-induced leukopenia. Although the patient had also received chloramphenicol and sulfisoxazole,[7] it is probable that the leukopenia was related to the TMCA treatment.

DISCUSSION

TMCA was selected for a clinical trial in a broad spectrum of malignant neoplasms on the basis of a preclinical study by Leiter et al (3). Their data indicated that TMCA had a therapeutic ratio that was better than that of any other colchicine analog tested. In our previous phase II trial (1), responses were noted in 19 of the 153 patients whose cases were evaluable. Because four of these responses occurred in patients with melanoma, the study was extended to include more patients with this disease. On the basis of the current data, however, it is concluded that TMCA has not proved to be clinically useful in the therapy for melanoma when given according to this dose schedule. Further trial of TMCA in the treatment of patients with lymphomas is in progress.

[7] Chloramphenicol: NSC-3069; acetamide, 2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]-.
Sulfisoxazole: NSC-13120; sulfanilamide, *N*1-(3,4-dimethyl-5-isoxazolyl)-.

REFERENCES

1. STOLINSKY, D. C., JACOBS, E. M., BATEMAN, J. R., ET AL. Clinical trial of trimethylcolchicinic acid methyl ether d-tartrate (TMCA; NSC-36354) in advanced cancer. Cancer Chemother Rep 51:25–34, 1967.

2. KARNOFSKY, D. A. Meaningful clinical classification of therapeutic responses to anticancer drugs. Clin Pharmacol Ther 2:709–712, 1961.

3. LEITER, J., DOWNING, V., HARTWELL, J. L., ET AL. Damage induced in Sarcoma 37 with chemical agents. III. Colchicine derivatives related to trimethylcolchicinic acid and to colchinol. J Natl Cancer Inst 13: 379–392, 1952.

Phase II Study of 5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) in Advanced Gastrointestinal Cancer [1,2,3]

C. G. Moertel, A. J. Schutt, R. J. Reitemeier, and R. G. Hahn [4]

SUMMARY

5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide was used in the treatment of 31 patients with advanced gastrointestinal cancer. Toxic reactions included nausea, vomiting, arm pain and phlebitis, tachycardia, facial flushing and paresthesia, urticaria, leukopenia, and thrombocytopenia. Cumulative bone marrow toxicity was noted also in several patients. There was no objective evidence of tumor response.

[Cancer Chemother Rep 56:267–269, 1972]

5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) is one of a variety of substituted triazeno imidazole carboxamides designed as inhibitors of purine synthesis. It has been shown to be active against a number of animal tumor systems and to produce complete inhibition of L1210 murine leukemia (1). Initial clinical trials using the oral route of administration (2) induced objective responses in lymphoma but none in a variety of other solid tumors and leukemias. Absorption from the gastrointestinal tract, however, was erratic.

Phase I studies with the more recently formulated parenteral preparation of NSC-82196 indicated that a suitable dosage plan for phase II trials would be 900 mg/m²/day for 5 days, to be repeated at 6-week intervals, with escalation of the daily dose of the drug to 1200 mg/m²/day if the initial course was well tolerated.[5] Our study was undertaken to evaluate NSC-82196 administered according to this plan to patients with gastrointestinal adenocarcinoma.

MATERIALS AND METHODS

Thirty-one patients with histologically confirmed metastatic adenocarcinoma known or presumed to have originated in the gastrointestinal tract were selected for study. The specific sites of origin are listed in table 1. Twenty patients were women and 11 were men. Their mean age was 61 years, with a range of 31–74 years. All were ambulatory outpatients and all were maintaining a reasonable state of nutrition prior to treatment. Thirteen patients had had no previous antineoplastic chemotherapy. Eighteen had been treated previously, primarily with 5-fluorouracil (NSC-19893) or camptothecin (NSC-100880). None of the patients had received any chemotherapeutic agent within 1 month of entry into the study, and all patients had normal leukocyte and platelet counts. Each patient had a measurable area of known cancer to serve as an objective indicator of response.

NSC-82196 was reconstituted in sterile water and injected immediately. The planned initial dose for all patients was 900 mg/m²/day for 5 days. One patient received only four doses because hematemesis occurred on Day 4 (*see*

[1] Received Oct 19, 1971; accepted Nov 8, 1971.
[2] Supported by contract NIH-70-2066 from Chemotherapy, National Cancer Institute (NCI), National Institutes of Health.
[3] NSC-82196 (TIC-mustard) was supplied by the Cancer Therapy Evaluation Branch, Chemotherapy, NCI.
[4] Division of Gastroenterology and Internal Medicine (Drs. Moertel, Reitemeier, and Schutt), and Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Clinic and Mayo Foundation, Rochester, Minn.
[5] Carter, S. K. Personal communication.

TABLE 1.—Sites of origin of primary gastrointestinal adenocarcinomas

Primary site	No. of patients
Large bowel	22
Pancreas	4*
Stomach	3
Bile duct	1
Liver (hepatoma)	1

*Two cases proved by pancreatic biopsy; 2 presumed on basis of clinical findings.

Toxic Reactions). White blood cell (wbc) counts were obtained twice weekly and platelet counts once weekly for at least 6 weeks after the start of therapy. If there was no objective change in the patient or if there was only minimal advancement of the disease, treatment was repeated at 6-week intervals. Five patients had repeat courses at the same dose level, four at doses escalated to 1200 mg/m²/day for 5 days, and two at a lower dose of 750 mg/m²/day for 5 days. Most patients received phenothiazine antiemetics to alleviate vomiting occurring during treatment.

RESULTS

Toxic Reactions

The toxic reactions experienced by patients during the first course of therapy are listed in table 2.

TABLE 2.—Toxic reactions to first course of therapy in 31 patients

Toxic reactions	No. of patients
Nonhematologic	
Nausea	30
Vomiting	24
Arm pain	21
Tachycardia	15
Arm vein phlebitis	9
Facial flushing or paresthesia	7
Chills	4
Urticaria	4
Hematemesis	2
Hematologic*	
Leukopenia (cells/mm³)	
<4000	9
<2000	2
Thrombocytopenia (cells/mm³)	
<150,000	12
< 50,000	1

*Counts could not be adequately followed in 2 patients who had early deaths.

Nausea, usually with vomiting, was an almost universal problem during therapy. In five patients this was experienced only on the first day of treatment, but in the other patients it occurred throughout treatment and was not well controlled by phenothiazine antiemetics. Hematemesis occurred in two patients who had severe vomiting. One of these patients had only minimal blood loss, but the other had severe and intractable hemorrhage causing death on the seventh day after the start of treatment. Necropsy revealed diffuse mucosal gastric bleeding resulting from erosive gastritis. There was also portal vein thrombosis which could have contributed to the blood loss problem.

Arm pain was troublesome for two thirds of the patients, and arm phlebitis developed in nine patients. Slow infusion of this drug could not be used to avoid this problem because of the danger of degradation of the drug, and flushing the vein after injection did not seem helpful.

Increasing tachycardia during the course of therapy was observed in 15 patients. Pretreatment pulse rates were increased by an average of 25 pulses/minute (range, 16–66 pulses/minute), with maximum pulse rates usually occurring on the third or fourth day of treatment. Less common reactions to treatment included facial flushing and paresthesia (occurring a few minutes after the injection had been given), urticaria (developing on Days 6–9), and shaking chills.

There was evidence of hematologic toxicity in 15 of 29 adequately observed patients, but the effects were generally mild. Two distinct nadirs of both leukocyte and platelet counts were observed. The first occurred between Days 8 and 13 and was very transient. The second nadir occurred on Day 26 (median; range, Days 17–52) for leukocytes and on Day 30 (median; range, Days 22–42) for platelets. Although this late hematologic depression usually persisted for only a very few days, in one patient the leukocyte count remained low for 65 days reaching a nadir of 1800 cells/mm³ on Day 52.

For three of the 11 retreated patients, there was evidence of definite compounding of hematologic toxicity with the later treatment courses. One patient had had a low wbc of 3400 cells/mm³ during 5 days of leukopenia with a first

course of the drug at 900 mg/m²/day × 5. When the same dose was repeated at 6 weeks, the wbc nadir was 1300 cells/mm³ and the duration of leukopenia was 34 days. A second patient, also retreated with the same course of 900 mg/m²/day × 5 after no hematologic toxicity had occurred with the first course, had 31 days of leukocyte depression with a wbc nadir of 2600 cells/mm³. A third patient, whose dose was reduced to 750 mg/m² for second and third courses, had progressive increases in duration of leukopenia from 4 to 15 to 22 days, and with the third course, he first had thrombocytopenia with the count reaching a nadir of 60,000 cells/mm³.

There was no discernible evidence of hepatic or renal toxicity.

Therapeutic Results

Therapeutic effect in patients receiving NSC-82196 was assessed 6 weeks after the start of therapy. At this time, four patients had remained objectively stable, two had died, and 24 had measurable advance of their disease. It was our subjective impression that several of these patients had demonstrated extraordinarily rapid progression of their cancer. For none of the patients who were retreated was there subsequent objective evidence of improvement.

No patient gained weight, and one third of the group lost 5% or more of their pretreatment weight. Three patients claimed improvement of their performance status, 11 were unchanged, and 17 claimed increasing disability.

DISCUSSION

With the exception of the higher incidence of arm pain and phlebitis, the toxicity spectrum of NSC-82196 was essentially the same as that observed earlier with the dimethyl analog, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) (3). Particularly disturbing with regard to possible clinical utility of these agents is their tendency to produce cumulative toxic effects in bone marrow tissue.

The dose of NSC-82196 used in this study was less than that which could have been tolerated with reasonable safety from a hematologic standpoint; on this basis, the adequacy of this phase II study is open to challenge. However, the severity of the nonhematologic side effects resulting from treatment with this dose would seem to place the regimen at the borderline of patient acceptance. It would seem reasonable to conclude that NSC-82196 is not an agent of potential value in the treatment of advanced gastrointestinal cancer.

REFERENCES

1. SHEALY, Y. F., and KRAUTH, C. A. Complete inhibition of mouse leukemia L1210 by 5(or 4)-[3,3-bis-(2-chloroethyl)-1-triazeno]imidazole-4(or 5)-carboxamide (NSC-82196). Nature 210:208–209, 1966.
2. VOGEL, C. L., DEVITA, V. T., DENHAM, C., ET AL. Preliminary clinical trials and clinical pharmacologic studies with 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (NSC-82196) given orally. Cancer Chemother Rep 55:159–165, 1971.
3. MOERTEL, C. G., REITEMEIER, R. J., HAHN, R. G., ET AL. Study of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in patients with gastrointestinal carcinoma. Cancer Chemother Rep 54:471–473, 1970.

ANNOUNCEMENTS

Tenth Symposium on Biomathematics and Computer Science in the Life Sciences

The University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, announces that the Tenth Symposium on Biomathematics and Computer Science in the Life Sciences will be held on March 29, 30, and 31, 1973, in Houston, Texas. Topics for the symposium will include mathematical, statistical, and computing applications in biology and medicine. Abstracts of all papers must be submitted to the Chairman, Dr. Stuart O. Zimmerman, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 6723 Bertner Drive, Houston, Texas 77025, prior to December 15, 1972.

For further information write to the Office of the Dean, The University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, PO Box 20367, Houston, Texas 77025.

One-Week Course in Cancer Chemotherapy: October 23–27, 1972

The Memorial Hospital for Cancer and Allied Diseases and Sloan-Kettering Institute for Cancer Research announce a 1-week course in cancer chemotherapy from October 23 to October 27, 1972.

This course will include lectures and panels on screening methods, pharmacologic techniques, methods for the clinical evaluation of potential chemotherapeutic agents, and a review of the chemistry and pharmacology of alkylating agents, antimetabolites, steroid hormones, and miscellaneous agents in the treatment of cancer. Discussions of specific problems in cancer will also be included. The course is principally designed for physicians interested in cancer chemotherapy.

Enrollment is limited, and applications should be submitted as soon as possible. The fee for the course is $150.00 payable at the time of application. Requests for application forms should be addressed to Dr. Irwin H. Krakoff, Memorial Hospital, 444 East 68th Street, New York, New York 10021.

A detailed outline of the course and further information will be mailed at a later date. Information regarding nearby hotels can be sent on request, but the Center cannot assume responsibility for making hotel reservations.

CORRECTION

Figure 2 in the article "Influence of Mitotic Cycle Inhibitors on the Antileukemic Activity of Cytosine Arabinoside (NSC–63878) in Mice Bearing Leukemia L1210" by S. Vadlamudi and A. Goldin (Vol 55, No. 5, p 552) was incorrectly printed. The following is the complete figure as it should have appeared.

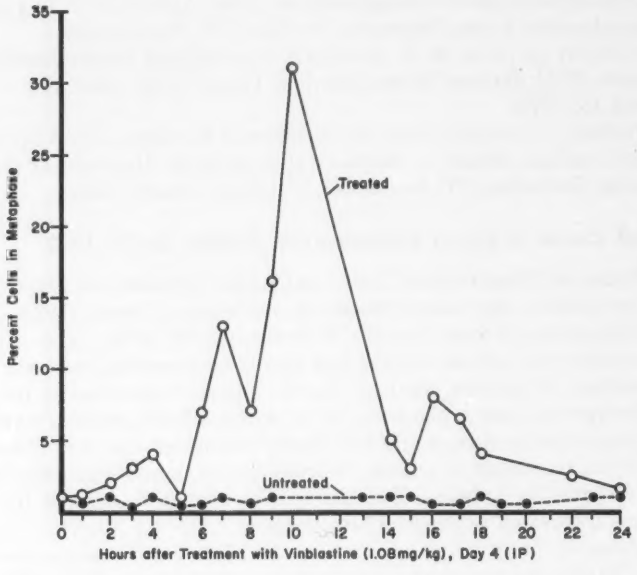

FIGURE 2.—Effect of in vivo treatment with vinblastine on leukemic cell cycle in mice bearing leukemia L1210.

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